16. Attention is also directed to *Ex parte Shirley*, (BPAI, 2009) Appeal No. 2009002352, which, at pages 21 and 26, states:

> The Specification's omission of the term "prior art" and inclusion of the prior-art disclaimer may initially appear to indicate that Appellants do not consider the single-step soft bake process to constitute prior art. To place these latter, contraindicative factors into the proper context though, we note that patent-application drafters regularly endeavor to avoid the indiscriminate or imprudent use of the descriptive label, "prior art." *See Riverwood Intern. Corp. v. R.A. Jones & Co., Inc.*, 324 F.3d 1346, 1354 (Fed. Cir. 2003) (citing *In re Fout*, 675 F.2d 297, 300 (CCPA 1982)) for the proposition that "section 102 is not the only source of ... prior art. Valid prior art may be created by the admissions of the parties"); *In re Nomiya*, 509 F.2d 566, 571 (CCPA 1975) (holding that an Applicant's labeling of certain figures as "prior art," *ipsissimis verbis*, constituted an admission that the pictured subject matter was prior art relative to Applicant's invention); MPEP § 21294 (instructing that "the examiner must determine whether the subject matter identified as 'prior art' is applicant's own work, or the work of another. In the absence of another credible explanation, examiners should treat such subject matter as the work of another").
>
> ***
>
> The Specification's omission of the term "prior art" and inclusion of the boilerplate prior-art disclaimer do not change our conclusion. In view of the record as a whole, these factors are ineffective in shielding Appellants from having their prior-art admissions treated as such.

17. Claims 82-84, 87-98, and 100 remain rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.).

18. Applicant, at page 2 of the as-filed disclosure, under "BACKGROUND OF THE INVENTION," teaches that various methods, including immunochemical assays for detecting human hemoglobin in fecal sample was known in the art. As stated therein:

> Fecal occult blood tests fall primarily into two categories, tests based on the use of chromogenic chemical reagents such as gum guaiac and immunochemical tests. The chemically based guaiac methods determine the presence of occult blood by the detection

> of the perioxidase activity of the hemoglobin in the blood present in the faecal sample. They require catalysis of peroxide into oxygen and water, and the subsequent oxidation of a colorless dye (most often into a colored form). However, peroxidase activity is also found in meats and vegetables. In order to produce accurate results, these tests require restriction of the intake of certain foods, drugs, vitamins, and other substances prior to and during the sample collection period. The sensitivity of the most commonly used guaiac FOBT (Hemoccult) is approximately 50%. Despite a specificity of 98%, the positive predictive value for FOBT is low. Methods of detecting occult blood based on porphyrin (heme and protpoporphyrin IX) analysis or immunologic tests using anti-hemoglobin antibodies improve on these results**. Immunochemical tests (FIT or iFOBT) that use anti- hemoglobin antibodies specific for human blood in extracts from stool do not require dietary restrictions;** however, they are more complicated and more expensive than peroxidase-based tests. (Emphasis added)

19. Applicant, page 5 of the disclosure, teaches that methods for detection of colorectal carcinoma based upon determining methylation of a number of different genes, including panels of genes, is disclosed in WO 2008/084219 A1 (Van England et al.).

> WO 2008/084219 relates to detection of colorectal cancer based upon determining methylation of a number of different genes, including panels of genes.

20. Applicant, at page 17, third paragraph, admits that methods for isolating DNA are known in the art. As asserted to therein:

> The methods of the invention may be carried out on purified or unpurified DNA-containing samples. However, in specific embodiments, DNA is isolated/extracted/purified from the sample. Any suitable DNA isolation technique may be utilized. Examples of purification techniques may be found in standard texts such as Molecular Cloning - A Laboratory Manual (Third Edition), Sambrook and Russell (see in particular Appendix 8 and Chapter 5 therein). (Emphasis added)

21. Applicant, at page 18, third paragraph, admits that kits for amplifying DNA in stool samples are commercially available. As admitted to therein:

> Amplification of DNA (using PCR) from natural sources is often inhibited by co-purified contaminants and various methods adopted for DNA extraction from environmental samples are available and provide an alternative for isolating DNA from faecal samples, according to specific embodiments of the invention. For instance, the QIAamp DNA Stool Mini Kit from QIAGEN adsorbs DNA-damaging substances and PCR inhibitors

present in the sample by InhibitEX. Other examples for application to faecal samples include the Wizard Genomic DNA Purification Kit (Promega), the NucliSENS® easyMAG TM (Biomerieux) and nucleic acid purification kits manufactured by Macherey Nagel.

22. Applicant, at page 19, further admits that kits for isolating DNA from fecal samples are commercially available. As asserted to therein:

> As discussed above, suitable commercially available kits, such as the QIAamp DNA Stool Mini Kit, may be employed in order to extract the DNA and remove potential inhibitors found in the faecal sample. This may involve processing smaller volumes of the overall faecal sample and pooling the resultant DNA in certain embodiments. For example, volumes of approximately 1 ml or less, such as approximately "750, 500, 250 or 100 µl may be used for DNA extraction. The resultant DNA from 2, 3, 4, 5 or more extractions may be pooled to give an overall DNA sample to be assessed according to the methods of the invention. DNA in the sample, pre- and/or post- pooling can be quantified as required, as discussed herein. (Emphasis added)

23. Applicant, at page 21, last paragraph, bridging to page 22 of the as-filed disclosure admits that numerous methods for assessing methylation are known in the art, including methods that comprise polymerase chain reaction (PCR), which is recognized as a form of amplifying DNA. Also admitted to therein is the use of chemical reagents so to modify the DNA prior to amplification was also well known in the art. As admitted to therein:

> **Determination of the methylation status may be achieved through any suitable means. Suitable examples include bisulphite genomic sequencing and/or by methylation specific PCR. Various techniques for assessing methylation status are known in the art and can be used in conjunction with the present invention**: sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR(McMS-PCR), MLPA with or without bisulphite treatment, QAMA (Zeschnigk et al, 2004), MSRE-PCR (Melnikov et al, 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulphite conversion-specific methylation-specific PCR (BS-MSP)(Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence diferences in PCR products of sodium bisulphite - treated DNA), methylation-sensitive single-nucleotide primer extension conformation(MS-SNuPE), methylation- sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulphite restriction analysis (McCOBRA)(Akey et al., 2002), PyroMethA, HeavyMethyl (Cottrell et al. 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional

> methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques for DNA methylation analysis is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264, Nature Reviews, 2003, Vol.3, 253-266; Oral Oncology, 2006, Vol. 42, 5- 13, which references are incorporated herein in their entirety. (Emphasis added)

24. Applicant, at page 24, third paragraph, admits that methods for performing MSP, wherein one employs primer pairs designed to distinguish methylated DNA from unmethylated DNA, was known in the art. As admitted to therein:

> In the MSP approach, DNA may be amplified using primer pairs designed to distinguish methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulphite treatment (**Herman et al., 1996; and WO 97/46705**). For example, bisulphite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. (Emphasis added)

25. The above showing is deemed to meet a limitation of claims 95, 96, and 100.

26. Applicant, at page 26, last paragraph, bridging to page 27, of the as-filed disclosure further asserts:

> Whilst PCR is the preferred nucleic acid amplification technique, other amplification techniques may also be utilised to detect the methylation status of the concerned gene. Such amplification techniques are well known in the art, and include methods such as NASBA (Compton, 1991), 3SR (Fahy et al., 1991) and Transcription Mediated Amplification (TMA). Other suitable amplification methods include the ligase chain reaction (LCR) (Barringer et al, 1990), selective amplification of target polynucleotide sequences (US Patent No. 6,410,276), consensus sequence primed polymerase chain reaction (US Patent No 4,437,975), arbitrarily primed polymerase chain reaction (WO 90/06995), invader technology, strand displacement technology, and nick displacement amplification (WO 2004/067726). This list is not intended to be exhaustive; any nucleic acid amplification technique may be used provided the appropriate nucleic acid product is specifically amplified. Thus, these amplification techniques may be tied in to MSP and/or bisulphite sequencing techniques for example. (Emphasis added)

27. A review of the disclosure fails to find where applicant has admitted that using a concentration of 10 ng/ml of hemoglobin in stool as a threshold value was known in the art.

28. Yee et al., paragraph [0041], teach setting a 10 ng/ml of hemoglobin in blood as a threshold value. Such a showing is deemed to meet a limitation of claims 87-94.

29. Neither the disclosure nor Yee et al., have been found to teach freezing a portion of a fecal sample.

30. Grow et al., abstract, teach the preparation of a fecal sample for immunoassay, which includes determining if human hemoglobin is present. As disclosed therein:

> A method for preparing a fecal sample for immunoassay testing comprising the steps of dispersing **a sample of from 1 to less than 10 wt. % of a stool sample** in an aqueous fecal test solution formulated with one or more preservatives, analyte stabilizing agents and endogenous interference reducing agents. The fecal solids are then permitted to settle to form a liquid phase substantially free from fecal solids, and the clarified liquid phase is removed to provide a test sample free from fecal solids. **The fecal test solutions contain suitable stool stabilizers such as buffers** and antimicrobial agents, analyte protecting agents such as proteolytic, reductive or oxidative enzyme inhibitors, endogenous assay interfering enzyme inhibitors such as a reducing agent, and non-specific binding inhibitors such as animal proteins. **The stool sample should be fresh or be fresh frozen** and thawed immediately before dispersion in the buffer solution. The sample is suitable for any solid-phase immunoassay determination of a fecal sample analyte. A method for determining analyte in the stool sample comprises conjugating anti-analyte antibody adhered to an insoluble support with analyte in the clarified sample, and determining the presence and extent of such conjugation. For determining human hemoglobin in a sample of human stool, the aqueous fecal test solution preferably contains a proteinase inhibitor, formaldehyde and an animal albumin. (Emphasis added)

31. The fact that only 1 to less than 10% of the sample is being used is deemed to meet a limitation of claim 82, step b). The fact that a buffer is being used is deemed to meet a limitation

of claim 82, step c). The fact that the sample can be frozen is deemed to meet a limitation of claim 83.

32. As evidenced above, Grow et al., teach preparation of a fecal sample for immunological testing, not analysis of nucleic acids contained therein.

33. Shuber et al., teach methods for stabilizing biological samples for nucleic acid analysis. As seen at paragraph [0009], the sample can be that of a patient's fecal sample. Shuber et al., paragraph [0031], teach:

> However, according to the invention, it may be particularly important to preserve sample DNA for purification, especially when looking for early indicia of diseases (e.g., indicia of adenomas or early stage cancers that may be present in less than about 1%, or about 0.1% or less of human genomes isolated from a stool sample). A common method to insure that DNA remains stable is to freeze stool samples as quickly as possible after collection, or to receive samples in centralized testing labs as quickly as possible.

34. Shuber et al., Example 2, teach the benefits of treating a stool sample with a nucleic acid stabilization solution. Such a showing is deemed to meet a limitation of claims 82 and 83.

35. As evidenced above, both Shuber et al., and Grow et al., teach freezing the sample, be it for immunological testing or analysis of the nucleotide sequence of a gene, and the methylation thereof.

36. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to analyze fecal samples for both blood (hemoglobin) as well as for a gene/sequence of interest. It would also have bene obvious to collect separate portion of a sample and to treat each in a manner that would either preserve the protein target(s) (Grow et al.) or the nucleic acid target(s) (Shuber et al.).

37. In view of the intense interest in detection of colorectal carcinoma in an individual, said ordinary artisan would have been amply motivated. And in view of the well-developed state of the art, and the fact that one would be using the disclosed methods for the same purpose, said ordinary artisan would have had a most reasonable expectation of success.

38. In view of the above analysis and in the absence of convincing evidence to the contrary, claims 82-84, 87-98, and 100 remain rejected under pre-AIA 35 U.S.C. §103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.).

Response to argument

39. Applicant's representative, at pages 2-5 of the response of 22 May 2020, hereinafter the response, traverses the rejection of claims 82-84, 87-98, and 100 under pre-AIA 35 U.S.C. §103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.). At page 4 of the response said representative asserts:

> The teachings of these references enumerated in the Office Action are related to immunoassay analysis of fecal samples for occult blood/hemoglobin (Yee and Grow) or for stabilizing samples for nucleic acid analysis (Shuber). For hemoglobin immunoassays, Yee and Grow teach methods for collecting, storing, processing, and testing fecal samples that are completely different than the methods taught by Shuber for nucleic acid analysis. Consequently, analyzing a fecal sample for both blood/hemoglobin and nucleic acid using the methods disclosed by Yee, Grow, and Shuber would result in a significant increase in work and cost compared to using any one of the methods alone.

40. The above argument has been considered and has not been found persuasive as applicant has not addressed all of the references relied upon. In particular, applicant has not addressed the teachings of the primary reference- applicant's own admissions as to what was known and how commercially-available kits can be used for the preparation of samples. It is further noted that

Yee et al., and grow et al., were not being relied upon for teaching a method of isolation. Rather, Yee et al., was being relied upon for teaching the setting of 10 ng/ml of hemoglobin in blood as a threshold value, and how such a teaching meets a limitation of claims 87-94. Grow et al., was being relied upon for teaching the freezing of a sample. Shuber et al., was relied upon for teaching methods for stabilizing biological samples for nucleic acid analysis wherein one uses a stabilizing buffer and can also freeze the sample.

41. While argument has been asserted that the amount of work required would be increased, no evidence has been provided to support this conclusion. To the contrary, and as noted above in paragraph 20, applicant asserts that "Any suitable DNA isolation technique may be utilized." (Emphasis added)

42. In view of the above analysis and in the absence of convincing evidence to the contrary, the rejection is maintained.

43. Claim 99 remains rejected under pre-AIA 35 U.S.C. §103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.) as applied to claims 82-84, 87-98, and 100 above, and further in view of WO 2004/083399 A2 (Goggins et al.).

44. See above for the basis of the rejection as it relates to the admissions of applicant and the teachings of Yee et al., Grow et al., and Shuber et al.

45. Neither admissions of applicant, nor the teachings of Yee et al., Grow et al., and Shuber et al., have been found to teach the genes listed in claim 99.

46. Goggins et al., in their abstract, teach:

> The present invention provides a method for detecting **pancreatic carcinoma** in a subject. The method includes contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of at least one gene or associated regulatory region of the gene and identifying aberrant methylation of regions of the gene or regulatory region, wherein aberrant methylation is identified as being different when compared to the same regions of the gene or associated regulatory region in a subject not having the pancreatic carcinoma, thereby detecting pancreatic carcinoma in the subject. (Emphasis added)

47. Goggins et al., at page 8, teach:

> Genes associated with pancreatic carcinoma are listed in Tables 1-3 by way of example. Specifically, such genes include CDH3, reprimo, CLDN5, DR3, **FOXE1**, LDOCl, LHXl, NEFH, NPTX2, PIGll, SARP2, ST14, SMARCAl, TJP2, UCHLl, WNT7A, or a combination thereof. (Emphasis added)

48. Goggins et al., at paragraph [0042] (page 16), teach that the cells to be tested can be obtained from the colon as well as from the pancreas. As stated therein:

> The test cell also can be **a component of a biological fluid**, for example, blood, lymph, cerebrospinal fluid, **pancreatic juice**, saliva, sputum, stool, urine, or ejaculate. If appropriate, the test cells also can be obtained by lavage, for example, for obtaining test cells from the **colon**, uterus, abdominal cavity, or the like, or using an aspiration procedure, for example, for obtaining a bone marrow sample. (Emphasis added)

49. Goggins et al., paragraph [0079], teach that the neoplasm can be that which occurs in the colon. As disclosed therein:

> A neoplasm creates an unstructured mass (a tumor) which can be either benign or malignant. For example, the neoplasm may be a head, neck, lung, esophageal, stomach, small bowel, **colon**, bladder, kidney, or cervical neoplasm. (Emphasis added)

50. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the teachings of applicant, Yee et al., Grow et al., and Shuber et al., whereby the gene to be evaluated in terms of methylation was FOXE1 as it had already been associated with cancer.

51. In view of the well-developed state of the art, and the specific guidance to evaluate the methylation of this specific gene as it had been associated with cancer, said ordinary artisan would have been amply motivated to have evaluated same, using known methods of sample collection, and analysis.

52. In view of the above analysis and in the absence of convincing evidence to the contrary, claim 99 remains rejected under pre-AIA 35 U.S.C. §103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.) as applied to claims 82-84, 87-98, and 100 above, and further in view of WO 2004/083399 A2 (Goggins et al.).

Response to argument

53. At page 5, bridging to page 6 of the response said representative traverses the rejection of claim 99 under §103(a). As asserted to therein:

> Nothing in Goggins suggests any utility in testing fecal samples using both blood/hemoglobin testing *and* nucleic acid testing for the FOXE1 gene for pancreatic cancer...

54. The above argument has been considered and has not been found persuasive for as noted above, Goggins et al., teach that the cell sample can be obtained from a variety of sources, including blood, pancreas, and colon. In view of the explicit guidance to obtain cells and materials from such a variety of sources, and to evaluate known informative genes for epigenetic modifications that are informative, said ordinary artisan would have been both amply motivated and would have had a most reasonable expectation of success.

55. At page 6 of the response said representative asserts:

> Furthermore, Goggins fails to cure the deficiencies of the Examiner's arguments regarding Yee, Grow, and Shuber set forth above and, for the same reasons, these arguments fail to establish the obviousness of claim 99 in view of the combination of Yee, Grow, Shuber and Goggins.

The above argument has been considered and has not been found persuasive towards the withdrawal of the rejection for as noted above, the rejection has been maintained.

56. In view of the above analysis and in the absence of convincing evidence to the contrary, claim 99 remains rejected under pre-AIA 35 U.S.C. §103(a) as being unpatentable over applicant's admissions in view of US 2008/0227208 A1 (Yee et al.), US 5,198,365 (Grow et al.), and US 2008/0124714 A1 (Shuber et al.) as applied to claims 82-84, 87-98, and 100 above, and further in view of WO 2004/083399 A2 (Goggins et al.).

Conclusion

57. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

a. US 2006/0084054 A1 (Alsobrook et al.), at paragraph [1531], teaches:

> Recently, **NDRG4**, another memember [*sic*] of Ndr family, was shown to be expressed in neurons of the brain and spinal cord. Its expression was markedly decreased in the brain of Alzheimer's disease patient (Zhou R H, Kokame K, Tsukamoto Y, Yutani C, Kato H, Miyata T. (2001) Characterization of the human NDRG gene family: a newly identified member, NDRG4, is specifically expressed in brain and heart. Genomics 73(1):86-97). Therapeutic modulation of this gene, expressed protein and/or use of antibodies or small molecule drugs targeting the gene or gene product are useful in the treatment of central nervous system disorders such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, schizophrenia and depression.

58. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

59. A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

60. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Bradley L. Sisson whose telephone number is (571)272-0751. The examiner can normally be reached on Monday to Thursday, from 6:30 AM to 5 PM..

61. Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

62. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

63. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR

system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Bradley L. Sisson/
Primary Examiner, Art Unit 1634

Notice of References Cited	Application/Control No. 15/634,607	Applicant(s)/Patent Under Reexamination Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20080227208-A1	09-2008	Yee; Hsiao-Ching	G01N33/72	436/66
*	B	US-5198365-A	03-1993	Grow; Michael A.	G01N33/528	436/17
*	C	US-20080124714-A1	05-2008	Shuber; Anthony P.	C12Q1/6806	435/6.16
*	D	US-20060084054-A1	04-2006	Alsobrook; John	C07K14/47	435/6.14
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
*	N	WO-2004083399-A2	09-2004	WO	Goggins et al.	C12Q1/6827
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

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CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020					
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

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	82	÷	✓	✓	✓					
	83	÷	✓	✓	✓					
	84	÷	✓	✓	✓					
	85	÷	-	-	-					
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	87	÷	✓	✓	✓					
	88	÷	✓	✓	✓					
	89	÷	✓	✓	✓					
	90	÷	✓	✓	✓					
	91	÷	✓	✓	✓					
	92	÷	✓	✓	✓					
	93	÷	✓	✓	✓					
	94	÷	✓	✓	✓					

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020					
	95	÷	✓	✓	✓					
	96	÷	✓	✓	✓					
	97	÷	✓	✓	✓					
	98	÷	✓	✓	✓					
	99	÷	✓	✓	✓					
	100	÷	✓	✓	✓					

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
				Filing Date	06/27/2017
				First Named Inventor	LOUWAGIE
				Art Unit	1634
				Examiner Name	SISSON
Sheet	1	of	2	Attorney Docket Number	EXCTD-35239.304

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] *(if known)*	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]

NONPATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
/B.L.S./		KIM et al., Noninvasive Molecular Biomarkers for the Detection of Colorectal Cancer. BMB Rep. 2008 Oct 31;41(10):685-92	
/B.L.S./		TURGEON et al., Fecal DNA-Based Detection of Colorectal Neoplasia. Curr Colorectal Cancer Rep. 2007 Oct;3(4):171-177	

Examiner Signature	/Bradley L. Sisson/	Date Considered	08/04/2020

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
				Filing Date	06/27/2017
				First Named Inventor	LOUWAGIE
				Art Unit	1634
				Examiner Name	SISSON
Sheet	2	of	2	*Attorney Docket Number*	EXCTD-35239.304

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Mary Ann D. Brow/	Date (YYYY-MM-DD)	2020-07-07
Name/Print	Mary Ann D. Brow	Registration Number	42363

.

PTO Notes regarding this form:

[1] Applicant's unique citation designation number (optional).
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[7] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.
[8] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**.

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CONFIRMATION NO. 1874

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/634,607	06/27/2017	435	1634	EXCTD-35239.304

APPLICANTS
EXACT SCIENCES DEVELOPMENT COMPANY, LLC, Madison, WI;

INVENTORS
Joost Louwagie, Dornach, SWITZERLAND;

**** CONTINUING DATA ***************************
This application is a CON of 15/010,436 01/29/2016 ABN
which is a CON of 13/147,570 03/12/2012 ABN
which is a 371 of PCT/GB2010/000180 02/03/2010
which claims benefit of 61/149,581 02/03/2009

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
07/12/2017

Foreign Priority claimed ❑ Yes ☑ No 35 USC 119(a-d) conditions met ❑ Yes ☑ No Verified and Acknowledged /BRADLEY L SISSON/ Examiner's Signature	❑ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		SWITZERLAND	0	~~19~~ 17	~~2~~ 1

ADDRESS
CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

TITLE
FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

FILING FEE RECEIVED		
1740	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	❑ All Fees ❑ 1.16 Fees (Filing) ❑ 1.17 Fees (Processing Ext. of time) ❑ 1.18 Fees (Issue) ❑ Other _____ ❑ Credit

BIB (Rev. 05/07).

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 19:10
S1	53	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:50
S2	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same "ng/ml" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:51
S3	9	(hemoglobin haemoglobin) with concentration same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:18
S4	260	DNA same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:35
S5	2	"20080227208"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:34
S6	1	"20080227208" and "10 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:35
S7	11	(hemoglobin haemoglobin) with "5 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:38
S8	0	NDGR4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR;	OR	OFF	2020/01/14 13:50

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S9	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:50
S10	6	NDRG4 same epigenetic and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:58
S11	24	"2006113770"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:02
S12	4	"2006113770" and NDRG4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:11
S13	6	NDRG4 same (epigenetic methylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S14	6	NDRG4 same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S15	0	"N-myc downstream regulated gene 4" same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S16	0	"N-myc downstream regulated gene 4" and (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S17	0	"N-myc downstream regulated gene 4" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:25

S18	1	"20060019256" and uptg	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:55
S19	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 15:35
S20	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:07
S21	3	fecal same (antibody antibodies immunoassay) same (DNA RNA polynucleotide "nucleic acid" gene) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:26
S22	2	(cancer carcinoma) same (PHACTR3 NDRG4 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFRP2 CDO1) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:40
S23	1	"200483399"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:42
S24	0	"200483399A2"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:43
S25	0	"200483399A2"	EPO	OR	OFF	2020/01/15 11:11
S26	0	"200483399"	EPO	OR	OFF	2020/01/15 11:11
S27	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 18:14

EAST Search History (Interference)

<This search history is empty>

8/4/2020 7:19:42 PM
C:\Users\bsisson\Documents\EAST\Workspaces\15634607.wsp

To: DOCKETING@CASIMIRJONES.COM,Genomic_docketing@cardinal-ip.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
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Aug 10, 2020 03:38:55 AM

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Application	Document	Mailroom Date	Attorney Docket No.
15634607	CTFR	08/10/2020	EXCTD-35239.304
	892	08/10/2020	EXCTD-35239.304
	1449	08/10/2020	EXCTD-35239.304

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Docket Number (Optional): EXCTD-35239.304

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Signature /Mary Ann D. Brow/

Typed or printed name Mary Ann D. Brow, Reg. No. 42363

In re Application of: LOUWAGIE

Application Number	Filed
15/634,607	27-Jun-2017

For: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Art Unit	Examiner
1634	SISSON

Applicant hereby **appeals** to the Patent Trial and Appeal Board from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 840

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I am the

☐ applicant

☑ attorney or agent of record
Registration number 42363

☐ attorney or agent acting under 37 CFR 1.34
Registration number _____

Signature /Mary Ann D. Brow/

Typed or printed name Mary Ann D. Brow

Telephone Number 608-662-1277

Date 11/10/2020

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Electronic Patent Application Fee Transmittal

Application Number:	15634607
Filing Date:	27-Jun-2017
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Filer:	Mary Ann Deese Brow/Stephanie Filandrinos
Attorney Docket Number:	EXCTD-35239.304

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
NOTICE OF APPEAL	1401	1	840	840
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**840**

Electronic Acknowledgement Receipt	
EFS ID:	41082896
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow/Stephanie Filandrinos
Filer Authorized By:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	10-NOV-2020
Filing Date:	27-JUN-2017
Time Stamp:	16:56:22
Application Type:	Utility under 35 USC 111(a)

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Notice of Appeal Filed	2020-11-10_35239-304_Not_Appeal.pdf	255260 a9d78aed3ffa2299c75d97756f48b28d24718405	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30708 01022fe414b5a5a996848377fa834821a6e107c1	no	2
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National Stage of an International Application under 35 U.S.C. 371
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

APPELLANT'S BRIEF

APPEAL NO.:

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF EFS WEB TRANSMISSION UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being transmitted to the United States Patent and Trademark Office transmitted via the Office electronic filing system in accordance with 37 C.F.R. §1.6(a)(4).

Dated: March 9, 2021 By: /Mary Ann D. Brow/
Mary Ann D. Brow, Reg. No. 42,363

This Brief supports the appeal to the Patent Trial and Appeal Board from the Final Office Action dated 08/10/2020 (hereinafter "Office Action") in the application identified above, and is in furtherance of the Notice of Appeal filed 11/10/2020, with Appellant's Brief due by 01/10/2021. A two-month extension of time, to 03/10/2021, is requested.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: **EXCTD-35239.304**. This paragraph is a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

This Brief contains these items under the following headings and in the order set forth below 37 C.F.R. § 41.37:

I. REAL PARTY IN INTEREST 3
II. RELATED APPEALS AND INTERFERENCES 3
III. SUMMARY OF CLAIMED SUBJECT MATTER 3
IV. ARGUMENT 7
V. CLAIMS APPENDIX 22

I. REAL PARTY IN INTEREST

The real party in interest is Exact Sciences Corp., Madison, WI., with subsidiary and Assignee of the application, Exact Sciences Development Company, LLC., Madison, WI.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to Appellant or Appellant's legal representative.

III. SUMMARY OF CLAIMED SUBJECT MATTER

Claims 1-81 and 85-86 were previously cancelled and claims 82-84 and 87-100 are pending, with claims 82 and 87 being independent. In response to the Office's requirement to elect one specific gene from claim 99, Appellant elected NDRG4.

The pending claims are directed to a method of processing a human fecal sample, *e.g.*, from a patient, in preparation for testing a single fecal sample for the presence of blood in the sample and also for the presence of epigenetic modification of DNA contained in the sample. (See, *e.g.*, the Specification of the instant application at page 28, first full paragraph.)

Claim 82 is directed toward a method of processing a fecal sample, the method comprising:

a) collecting a fecal sample from a human subject; (*see, e.g.,* Specification, page 32 at line 5)

b) removing a portion of said fecal sample to produce a removed portion and a remaining portion of said fecal sample; *(see, e.g., id.* at page 5, last full paragraph, and page 28, second full paragraph)

c) combining the removed portion with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample; *(see, e.g., id.* at page 5, last full paragraph, and page 28, second full paragraph)

d) stabilizing the remaining portion of the fecal sample. *(see, e.g., id.* at page 5, last full paragraph, and page 28, second full paragraph)

Claim 83 is directed to the method of claim 82, wherein stabilizing of the remaining portion of the fecal sample comprises one or both of:

i) adding a stabilizing buffer to said remaining sample; *(see, e.g., id.* at page 28, second full paragraph

ii) freezing said remaining sample. *(see, e.g., id.* at page 18, last paragraph)

Claim 84 is directed to method of claim 82, further comprising delivering the removed portion and the remaining portion of the fecal sample to a medical diagnostics laboratory. *(see, e.g., id.* at page 28, last paragraph)

85-86 were previously cancelled.

Claim 87 is directed to a method of processing a fecal sample, the method comprising:

A) obtaining a pair of portions of a fecal sample from a human subject, the pair of portions comprising a removed portion and a stabilized remaining portion of a fecal sample obtained by the method of claim 82, and

B) testing the removed portion of said fecal sample for the presence of blood. *(see, e.g., id.* at page 28, last paragraph)

Claim 88 is directed to the method of claim 87, wherein said testing comprises testing the removed portion of said fecal sample for a concentration of hemoglobin present in said removed portion. *(see, e.g., id.* at page 12, first full paragraph)

Claim 89 is directed to the method of claim 87, wherein testing for the concentration of hemoglobin in said removed portion comprises immunochemical detection of hemoglobin. *(see, e.g., id.* at page 12, first full paragraph)

Claim 90 is directed to the method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 5 ng/ml. *(see, e.g., id.* at paragraph bridging page 13-

14)

Claim 91 is directed to the method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 10 ng/ml. *(see, e.g., id.* at paragraph bridging page 13-14)

Claim 92 is directed to the method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 20 ng/ml. *(see, e.g., id.* at paragraph bridging page 13-14)

Claim 93 is directed to the method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 50 ng/ml. *(see, e.g., id.* at paragraph bridging page 13-14)

Claim 94 is directed to the method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 200 ng/ml. *(see, e.g., id.* at paragraph bridging page 13-14)

Claim 95 is directed to the method claim 87, further comprising:

C) extracting DNA from the stabilized remaining portion of said fecal sample *(see, e.g., id.* at page 17, last full paragraph) and

D) analyzing said DNA for the presence of DNA from a human, wherein the DNA from a human has an epigenetic modification. . *(see, e.g., id.* at page 14, first full paragraph)

Claim 96 is directed to the method of claim 95, wherein said epigenetic modification comprises aberrant methylation. *(see, e.g., id.* at page 20, second paragraph)

Claim 97 is directed to the method of claim 95, wherein said aberrant methylation comprises hypermethylation. *(see, e.g., id.* at page 20, second paragraph)

Claim 98 is directed to the method of claim 95, wherein said DNA from a human comprises a gene and/or a promoter region of a gene. *(see, e.g., id.* at page 20, second paragraph)

Claim 99 is directed to the method of claim 98, wherein said gene selected from the group consisting of PHACTR3, NDRG4, FOXE1 , GATA4, GPNMB, TFPI2, SOX17, SYNE1 , LAMA, MMP2, OSMR, SFRP2, and CDO1. *(see, e.g., id.* at page 37, third paragraph)

Claim 100 is directed to the method of claim 96, wherein analyzing said DNA comprises modifying said DNA with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil. *(see, e.g., id.* at page 23, second paragraph)

IV. ARGUMENT

The Argument is set forth in the following sections:

A. Grounds of rejection to be reviewed;

B. Summary of the technology and arguments;

C. Summary of teachings cited and characterized by the Office;

D. Applicable law; and

E. Detailed argument.

A. Grounds of rejection to be reviewed

i. Whether the Office erred in rejecting claims 82-84 and 87-100 under pre-AIA 35 U.S.C. §103(a) as being unpatentable over Appellant's alleged admissions in view of US 2008/0227208 A1 ("Yee"); US 5,198,365 ("Grow"); and US 2008/0124714 A1 ("Shuber").

ii. Whether the Office erred in rejecting claim 99 as being unpatentable over Appellant's alleged admissions in view of Yee, Grow, and Shuber, as applied to claim 82, further in view of WO 2004/083399 A2 ("Goggins").

B. Summary of the technology and arguments

The instant claims are directed to a method of collecting a fecal sample from a subject and dividing the collected sample into portions that are separately stabilized, *e.g.*, for detection of different types of analytes in each portion. In particular embodiments, one portion is treated to stabilize blood proteins and another portion is separately treated to stabilize nucleic acid, *e.g.*, DNA. A fecal sample collected in the claimed manner is suitable for detecting blood proteins in one portion and DNA in the other portion of the same fecal sample.

The Office has erred in finding the claims obvious for at least the following reasons:

1) The Office has mischaracterized the teachings of Grow, and all rejections rely on conclusions drawn from this mischaracterization;

2) The combination of references fails to teach all the features of the invention as claimed; and

3) The Office relied on conclusory statements to support the rejection and has failed to provide articulated reasoning with proper rational underpinning to support the legal conclusion of obviousness, and has relied on improper hindsight reconstruction to arrive at Appellant's claims.

C. Summary of teachings cited and characterized by the Office

Appellant's Specification ("Appellant's alleged admissions")

In making the rejections, the Office starts with Appellant's alleged admissions as the primary basis for the rejections. The Office points to Appellant's Specification as allegedly teaching the following list of features:

- That immunochemical assays for detecting human hemoglobin in fecal sample were known in the art (Office Action, ¶18);
- That detection of colorectal carcinoma based upon determining methylation of a number of different genes is disclosed in is disclosed in WO 2008/084219 Al (Van England et al.) (Office Action, ¶19);
- That methods for isolating DNA are known in the art. (Office Action, ¶20);
- That kits for amplifying DNA in stool samples are commercially available (Office Action, ¶21);
- That kits for isolating DNA from fecal samples are commercially available. (Office Action, ¶22);
- That numerous methods for assessing methylation are known in the art, including methods that comprise polymerase chain reaction (PCR), which is recognized as a form of amplifying DNA. Also admitted to therein is the use of chemical reagents so to modify the DNA prior to amplification was also well known in the art. (Office Action, ¶23);
- That methods for performing MSP [methylation-specific PCR], wherein one employs primer pairs designed to distinguish methylated DNA from unmethylated DNA, was known in the art. (Office Action, ¶24); and

– That:

> Whilst PCR is the preferred nucleic acid amplification technique, other amplification techniques may also be utilised to detect the methylation status of the concerned gene. Such amplification techniques are well known in the art, and include methods such as NASBA (Compton, 1991), 3SR (Fahy et al., 1991) and Transcription Mediated Amplification (TMA). Other suitable amplification methods include the ligase chain reaction (LCR) (Barringer et al, 1990), selective amplification of target polynucleotide sequences (US Patent No. 6,410,276), consensus sequence primed polymerase chain reaction (US Patent No 4,437,975), arbitrarily primed polymerase chain reaction (WO 90/06995), invader technology, strand displacement technology, and nick displacement amplification (WO 2004/067726). This list is not intended to be exhaustive; any nucleic acid amplification technique may be used provided the appropriate nucleic acid product is specifically amplified. Thus, these amplification techniques may be tied in to MSP and/or bisulphite sequencing techniques for example. (Emphasis added)

(Office Action, ¶26).

The Office admits that Appellant's disclosure does not teach that using a concentration of 10 ng/ml of hemoglobin in stool as a threshold value or freezing a portion of a fecal sample were know in the art (Office Action, ¶¶27 and 29.)

Cited secondary references

In making the rejections, the Office further points to four secondary references: Yee, Grow, Shuber, and Goggins.

1. The Office points to Yee as teaching setting a 10 ng/ml of hemoglobin in blood as a threshold value (Office Action, ¶¶28 and 40.)

2. The Office points to Grow as teaching using 1 to less than 10% of a sample preparation of a fecal sample for immunoassay, and for freezing a sample (Office Action, ¶¶ 30-32, and 40.)

3. The Office points to Shuber as teaching treating a stool sample with a nucleic acid stabilizing solution for nucleic acid analysis and for freezing a sample. (Office Action, ¶¶33-35 and 40.)

4. The Office points to Goggins as teaching testing FOXE1 in association with pancreatic carcinoma, and that neoplasm can occur in the colon. (Office Action, ¶¶ 46-49.)

D. Applicable Law

A proper obviousness determination requires that an Office make "a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art." *See In re Wada and Murphy*, Appeal 2007-3733, *citing In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis in original). Further, the necessary presence of all claim features is axiomatic, since the Supreme Court has long held that obviousness is a question of law based on underlying factual inquiries, including … ascertaining the differences between *the claimed invention* and the prior art. *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966) (emphasis added). Indeed, Section 904 of the MPEP instructs Examiners to conduct an art search that covers "the invention *as described and claimed*." In short, it remains well-settled law that obviousness requires at least a suggestion of all of the features in a claim. *See In re Wada and Murphy*, *citing CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) and *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)).

MPEP § 2143 points to the Supreme Court in *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727 (2007) as stating that "[r]ejections on obviousness grounds **cannot be sustained by mere conclusory statements**; instead, **there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness**." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (*quoting In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)(emphasis added).

It has further been confirmed by the Board in *In re Nuvasive, Inc.*, 842 F.3d 1376 (2016), that "**conclusory statements" alone are insufficient** and, instead, the finding **must be supported by a "reasoned explanation**." (*In re Nuvasive*, Inc., 842 F.3d 1376,1383 (2016) citing *Lee*, 277 F.3d at 1342, 1345. Further, "references to 'common sense' ... cannot be used as a wholesale substitute for reasoned analysis and evidentiary support...." (*Id.*, citing *Arendi S.A.R.L. v. Apple Inc.*, 832 F.3d 1355, 1362 (Fed. Cir. 2016)).

In presenting the *prima facie* case, it is well established that deriving the claimed technology through *ex post* hindsight reasoning is impermissible. In particular, "**[i]t is**

impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 972 F.2d 1260, 1266, (Fed. Cir. 1992); *ATD Corp. v. Lydall, Inc.*, 159 F.3d 534, 546 (Fed. Cir. 1998), emphasis added. ("obviousness cannot be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention".)

Consequently, an examiner absolutely must avoid using hindsight, and the legal conclusion must be reached on the basis of the facts gleaned from the prior art. See MPEP § 2142. At the outset, hindsight must not be used to define the problem that the invention solves. *Mintz v. Dietz & Watson, Inc.*, 679 F.3d 1372 (Fed. Cir. 2012). Then, hindsight must not be used to gather or suggest the elements of the invention. See *In re Ochiai*, 71 F.3d at 1572 (Fed. Cir. 1995). Finally, "both the suggestion and the expectation of success must be founded in the prior art, not in the Appellant's disclosure." *In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988).

E. Detailed argument

1) The Office has mischaracterized the teachings of Grow, and all rejections rely on conclusions drawn from the mischaracterization

The Office highlights the following passage from the Abstract of Grow:

> 30. Grow et al., abstract, teach the preparation of a fecal sample for immunoassay, which includes determining if human hemoglobin is present. As disclosed therein:
>
> A method for preparing a fecal sample for immunoassay testing comprising the steps of dispersing **a sample of from 1 to less than 10 wt. % of a stool sample** in an aqueous fecal test solution formulated with one or more preservatives, analyte stabilizing agents and endogenous interference reducing agents. The fecal solids are then permitted to settle to form a liquid phase substantially free from fecal solids, and the clarified liquid phase is removed to provide a test sample free from fecal solids. **The fecal test solutions contain suitable stool stabilizers such as buffers** and antimicrobial agents, analyte protecting agents such as proteolytic, reductive or oxidative enzyme inhibitors, endogenous assay interfering enzyme inhibitors such as a reducing agent, and non-specific binding inhibitors such as animal proteins. **The stool sample should be fresh or be fresh frozen** and thawed immediately before dispersion in the buffer solution. The sample is suitable for any solid-phase immunoassay determination of a fecal sample analyte. A method for determining analyte in the stool sample comprises conjugating anti-analyte antibody adhered to an insoluble support with analyte in the clarified sample, and determining the presence and extent of such conjugation. For determining human hemoglobin in a sample of human stool, the aqueous fecal test solution preferably contains a proteinase inhibitor, formaldehyde and an animal albumin. (Emphasis added)

(Office Action, ¶30).

Citing this passage, the Office then asserts that "The fact that only 1 to less than 10% of the sample is being used is deemed to meet a limitation of claim 82, step b)." (see Office Action at ¶31).

The Office has mischaracterized what Grow plainly says. Grow does not say 1 to less than 10% [of the sample] but recites "1 to less than 10 **wt.** %", which has an entirely different meaning. "**Wt.%**" refers not to a fraction of the sample. It is a standard abbreviation for "weight percent," or the weight or mass fraction of one component, especially a solid (*e.g.*, stool sample) relative to the total weight or mass of a solution or suspension (*e.g.*, Grow's aqueous fecal test solution with dispersed stool sample in it). Read accurately and in its entirety, the Abstract of Grow describes a preparation of fecal material dispersed in an "aqueous fecal test solution" in which the stool sample is "from 1 to less than 10 wt. %" of the total composition, with the balance of the weight (from 99% to greater than 90%) being the weight of the aqueous fecal test

solution. The term "wt. %" is used consistently throughout Grow and has nothing to do with a subportion of a fecal sample.

Grow says nothing about using *only a portion* of a fecal sample, nor is partitioning of a fecal sample inherent in, or required by, the method of Grow. For example, taking the weight of 100% of *any* size of fecal sample as a first part, the skilled artisan readily understands adding more than 9 parts of aqueous fecal test solution to arrive at a mixture that is less than 10 wt.% stool sample in the resulting dispersed sample preparation, with no need to remove any portion of the fecal sample.

Grow thus fails to provide the limitation of claim 82, step b).

The Office also points to the Abstract of Grow above as teaching that "the sample can be frozen," and alleges that this meets the limitation of claim 83. (Office Action, ¶31.)

However, claim 83 depends from claim 82, and the freezing step recited in claim 83 applies only to the "remaining portion" of the fecal sample that is produced as set forth in claim 82, step b), *i.e.*, by first removing the "removed portion" of the fecal sample. Grow's mention that a "stool sample should be fresh or frozen" makes no mention of partitioning a fecal sample, and does not teach either producing a "remaining portion" from a fecal sample as set forth in claim 82, step b), or freezing the remaining portion after removal of the removed portion.

Grow does not and cannot meet the limitations of claim 83 because Grow fails to meet the limitations of claim 82.

In rejecting each of claims 82-84 and 87-100, the Office relies *entirely* on this mischaracterization of Grow for a teaching of the limitation of claim 82, step b), as described above. Each and every one of Appellant's pending claims includes this element, by dependence from or reference to claim 82. Thus, the rejection of each of the claims relies on the Office's erroneous reading of Grow. The Office has erred in making the rejections for at least this reason.

2) The combination of references fails to teach all the features of the invention as claimed

Each and every pending claim includes the limitation of claim 82, step b), as described above, by dependence from or reference to claim 82. Neither Appellant's alleged admissions, nor

any of Yee, Shuber, or Goggins teach the limitation of claim 82, step b), so do not cure the deficiency.

Further, each and every claim includes the pair of separately stabilized portions produced by the method recited in claim 82: a removed portion and a remaining portion from the same fecal sample.

Appellant's alleged admissions do not include these features, nor do the secondary references enumerated in the Office Action teach them. The secondary references are directed to immunoassay analysis of fecal samples for occult blood/hemoglobin (Yee and Grow) **or** for stabilizing fecal samples for nucleic acid analysis (Shuber). Neither the Appellant's alleged admissions nor secondary references Yee, Grow, or Shuber teach or suggest testing for both occult blood/hemoglobin and nucleic acid in the same fecal sample. Thus, it is not surprising that none of the references cited by the Office, taken alone or in any combination, teach or suggest producing a removed portion and a remaining portion from the same fecal sample, and separately stabilizing the pair of portions, as set for in claim 82 and the remaining claims. Goggins, cited as teaching, *inter alia*, methylation of the FOXE1 gene (a gene listed in Appellant's claim 99) in association with pancreatic carcinoma (Office Action, ¶¶46-50), does not cure the deficiency.

The combination of references thus fails to teach all the elements of claims 82-84 and 87-100, and does not establish *prima facie* obviousness of these claims. The Office has therefore further erred in making the rejections for at least this reason.

3) The Office relied on conclusory statements and has failed to provide articulated reasoning with proper rational underpinning to support the legal conclusion of obviousness, and has relied on improper hindsight reconstruction to arrive at Appellant's claims

As discussed above, "[r]ejections on obviousness grounds **cannot be sustained by mere conclusory statements**; instead, **there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness**." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007). Further, "**[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious.**" *In re Fritch*, 972 F.2d 1260, 1266, (Fed. Cir.

1992); *ATD Corp. v. Lydall, Inc.*, 159 F.3d 534, 546 (Fed. Cir. 1998).

Regarding claims 82-84 and 87-100, in making the rejections of these claims, the Office states that "In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to analyze fecal samples for both blood (hemoglobin) as well as for gene/sequence of interest. (Office Action, ¶36).

This statement is entirely unsupported by evidence or argument. There is no "above showing" in the Office Action that contains any suggestion to analyze fecal samples for both blood (hemoglobin) as well as for gene/sequence of interest.

The summary of teachings in part C of this paper, above, lists the teachings and limitations to which the Office points in characterizing Appellant's alleged admissions, and in characterizing Yee, Grow, Shuber, and Goggins. As discussed above, none of the references cited by the Office, taken alone or in any combination, teach or suggest producing a removed portion and a remaining portion from the same fecal sample, and separately stabilizing the pair of portions, and the Office has failed to provide any proper rationale for modifying the teachings of the references to arrive at Appellant's claims.

The Office has enumerated the teachings of Yee and Grow with respect to testing samples for blood, and has not identified, for example, any deficiencies of blood/hemoglobin testing of fecal samples as taught by Grow and Yee that are curable by the addition of Shuber's DNA testing. Conversely, the Office has pointed to Shuber as teaching the benefits of treating a stool sample with a nucleic acid stabilization solution (Office Action, ¶¶33-34), but has not identified any deficiencies of DNA testing of fecal samples as taught by Shuber that are curable by addition of Yee and Grow's blood/hemoglobin testing. The Office has pointed to nothing in these references that suggests that any benefit or improvement that reasonably could be expected by subjecting a single fecal sample to both types of tests.

Office further states that "It would also have bene [sic] obvious to collect separate portion of a sample and to treat each in a manner that would either preserve the protein target(s) (Grow et al.) or the nucleic acid target(s) (Shuber et al.)." (Office Action, ¶36).

This statement is also unsupported by evidence or reasoned argument. As discussed above, the Office has provided no evidence suggesting that combining a test for a blood protein target and a test for a nucleic acid target from a single fecal sample would or might provide any kind of benefit or improvement. Having failed to provide any reason for the skilled artisan to subject a single fecal sample to both types of tests, the Office has also failed to provide any rationale for the invention in the embodiment as claimed, *i.e.*, for producing a removed portion and a remaining portion from the same fecal sample, and separately stabilizing the pair of portions, as set forth in claim 82.

The Office's sole argument in support for combining the references consists of the following statement:

> 37. In view of the intense interest in detection of colorectal carcinoma in an individual, said ordinary artisan would have been amply motivated. And in view of the well-developed state of the art, and the fact that one would be using the disclosed methods for the same purpose, said ordinary artisan would have had a most reasonable expectation of success.

(Office Action, ¶37)

In pointing to "the intense interest in detection of colorectal carcinoma in an individual" as a basis for asserting that the skilled person would be motivated to combine the references and to modify and piece together their teachings to arrive at Appellant's claimed embodiment, the Office has failed to articulate precisely what the skilled person would be seeking to achieve by combining the references, *e.g.*, how making the combination would serve to address "the intense interest in detection of colorectal carcinoma in an individual."

As noted above, the Office has not identified a single deficiency in any of the cited art nor a single benefit conferred by combining the teachings to motivate the skilled person to combine the references. Nothing in these references suggests performing both testing methods on a single fecal specimen might offer any particular improvement or benefit over the use of one testing method alone.

Further, as discussed above, *each and every pending claim* includes the pair of portions of a fecal sample: a removed portion and a remaining portion from the same fecal sample,

separately stabilized. The pair of portions is produced by the method recited in claim 82. Even if some advantage of using both the blood and DNA testing on feces from a subject is postulated, the Office has provided no reasoning or rational as to why the skilled person would be motivated to apply the different tests from the cited art on the same fecal sample.

Rather, the skilled person, considering the cited art, is left to guess at whether expending the time and effort to conduct both blood/hemoglobin testing and nucleic acid testing on a single fecal sample *might* produce some beneficial result, rather than merely producing redundant diagnostic information.

Although the Office asserts that the skilled person would have been amply motivated to make the combination by "intense interest in detection of colorectal carcinoma in an individual," the Office has not shown why, based on the cited art and Appellant's alleged admission, a skilled person would believe that testing a fecal sample using both blood/hemoglobin testing *and* DNA testing would be expected to offer any benefit with respect to detection of colorectal carcinoma at all, much less a benefit of sufficient importance or magnitude to offset the increase in costs and labor of running both of these different tests on each sample, as compared to running a single type of test on the sample. (The Office asserts at ¶41 of the Office Action that Appellant has provided no evidence to support that the amount of work would be increased by performing both tests, but it is objectively factual that performing one test is less work than performing that test, plus performing a second, different test on a separate portion of the sample.)

As discussed above, "It is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 972 F.2d 1260, 1266, (Fed. Cir. 1992); *ATD Corp. v. Lydall, Inc.*, 159 F.3d 534, 546 (Fed. Cir. 1998). "[H]indsight" is inferred when the specific understanding or principal within the knowledge of one of ordinary skill in the art leading to the modification of the prior art in order to arrive at appellant's claimed invention has not been explained." *In re Rouffet*, 149 F.3d 1350, 1358 (Fed. Cir. 1998).

Appellant's Specification teaches the benefits of testing a fecal sample for both blood proteins (referenced as fecal immunoassay testing, or "FIT" testing) and DNA marker modification. See, *e.g.*, Example 1 starting on page 43. For example, for fecal samples in sample set A, Table 3 on page 49 show sensitivities of 52% to 70% for samples tested for

methylation of the combination of NDRG4, FOXE1, and PHACTR marker genes, and Table 4 on the same page shows sensitivity of 78% by FIT testing the same set A of fecal samples. Table 6, bridging pages 50-51, shows the results of the combining these test methods, with sensitivities raging from 91% to 96% with the combined testing on sample set A.

In the context of the technology disclosed by Appellant, there is the requirement for appropriate sample processing. To meet this requirement, Appellant's technology provides a method of sample processing comprising removing a portion of a collected fecal sample and adding the removed portion of the sample to a buffer which prevents denaturation or degradation of blood proteins found in the fecal sample, and separately stabilizing the remaining sample, *e.g.*, to preserve DNA in the remaining portion. One particular benefit is that a fecal sample may be collected and partitioned into separately stabilized portions at home by the subject, *e.g.*, prior to delivery to a laboratory. (See, *e.g.*, the Specification at page 28, lines 1-7 of the second full paragraph.)

Given the absence of any suggestion of partitioning fecal samples in the art cited by the Office, it can only be concluded that the Office has done precisely what is forbidden, and has used the disclosure of Appellant's Specification and claims as a guide to selectively cull from the prior art elements that fit the parameters of the claims, using Appellant's disclosure as a template to piece together the embodiments as claimed. The Office has erred in making the rejections for at least this reason.

Regarding Claim 99, the Office cites Appellant's alleged admissions, Yee, Grow, and Shuber as applied to claims 82-84, 87-98, and 100, further in view of Goggins.

The Office admits that the combination of Appellant's alleged admissions, Yee, Grow, Shuber does not teach the genes listed in claim 99. (Office Action, ¶45). The Office points to Goggins as teaching, *inter alia,* the FOXE1 mutation in association with pancreatic carcinoma (Office Action, ¶47).

The Office asserts that "it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the teachings of applicant, Yee et al., Grow et al., and Shuber et al., whereby the gene to be evaluated in terms of methylation was FOXEl as it had already been associated with cancer." (Office Action, ¶50).

However, the *pancreatic* cancer of Goggins is the wrong cancer to support the Office's argument for the alleged obviousness of combining Appellant's alleged admissions, Yee, Grow, Shuber. The Office fails to explain how Goggins's disclosure of the FOXE1 marker in association with *pancreatic* cancer might point the skilled person to process a fecal sample in the manner of the instant claims. Notably, Goggins does not disclose fecal samples as a source of cells to be tested. (see Goggins at ¶0042, as cited in Office Action at ¶48). Nothing in Goggins suggests any utility in testing fecal samples, much less testing the same fecal sample using both blood/hemoglobin testing *and* nucleic acid testing for the FOXE1 gene for *pancreatic* cancer (or any other cancer).

Still further, Goggins' teaching of FOXE1 as a useful marker for *pancreatic* carcinoma does not in any way suggest a utility in line with the "intense interest in detection of *colorectal* carcinoma in an individual," which is the sole motivation offered by the Office for combining the Appellant's alleged admissions, Yee, Grow, and Shuber. To the contrary, if, as the Office has argued, detection of the methylation status of the FOXE1 gene in a sample collected from a colon is indicative of *pancreatic* carcinoma, then the skilled person considering the teachings of Goggins *would exclude* FOXE1 for detecting *colorectal* carcinoma by analysis of such samples, as Goggins teaches that detecting methylation of FOXE1 in a sample is indicative of a different type of carcinoma. The Office has improperly relied on Appellant's own Specification for the teaching to test FOXE1 DNA in a stabilized remaining portion of a fecal sample in association with detecting *colorectal* cancer, and further in association with testing a removed portion of the same fecal sample for occult blood/hemoglobin.

The Office asserts that Goggins' teaches that "the neoplasm" can be that which occurs in the colon (Office Action, ¶49, citing Goggins at ¶0079), seemingly suggesting that Goggins links methylation of FOXE1 with colorectal carcinoma. The Office again misrepresents the teachings of the cited reference. Paragraph 0079 of Goggins provided a *general* definition of "a neoplasm" as creating an unstructured mass or tumor, and that such a neoplasm "may be a head, neck, lung, esophageal, stomach, small bowel, colon, bladder, kidney, or cervical neoplasm":

> **[0079]** A cell proliferative disorder as described herein may be a neoplasm. Such neoplasms are either benign or malignant. The term "neoplasm" refers to a new, abnormal growth of cells or a growth of abnormal cells that reproduce faster than normal. A neoplasm creates an unstructured mass (a tumor) which can be either benign or malignant. For example, the neoplasm may be a head, neck, lung, esophageal, stomach, small bowel, colon, bladder, kidney, or cervical neoplasm. The term "benign" refers to a tumor that is noncancerous, e.g. its cells do not proliferate or invade surrounding tissues. The term "malignant" refers to a tumor that is metastastic or no longer under normal cellular growth control.

(Goggins, ¶0079, emphasis added)

Read in context, it is clear that Goggins is teaching that neoplasms *in general* may occur in any of these sites. This paragraph in no way suggests any link between colorectal neoplasm and methylation of the FOXE1 gene.

Lastly, as discussed above, Goggins fails to cure the deficiencies of the Office's arguments regarding Appellant's alleged admissions, Yee, Grow, and Shuber as applied to claim 82, as nothing in Goggins teaches or suggests testing for both occult blood/hemoglobin and methylation of FOXE1 in the same fecal sample, nor a method of partitioning and separately stabilizing the portions of the fecal sample, as set forth in claim 82.

CONCLUSION

In view of the points discussed above, it is clear that the Office has erred and has failed to establish *prima facie* obviousness of claims 82-84 and 87-100 over Appellant's alleged admissions in view of Yee, Grow, and Shuber, with or without Goggins, and that these rejections should be reversed.

Respectfully submitted,

Dated: March 9, 2021 /Mary Ann D. Brow/

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V. CLAIMS APPENDIX

1-81. (canceled)

82. A method of processing a fecal sample, the method comprising

a) collecting a fecal sample from a human subject;

b) removing a portion of said fecal sample to produce a removed portion and a remaining portion of said fecal sample;

c) combining the removed portion with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample;

d) stabilizing the remaining portion of the fecal sample.

83. The method of claim 82, wherein said stabilizing of said remaining portion of the fecal sample comprises one or both of:

i) adding a stabilizing buffer to said remaining sample;

ii) freezing said remaining sample.

84. The method of claim 82, further comprising delivering the removed portion and the remaining portion of the fecal sample to a medical diagnostics laboratory.

85-86. (canceled)

87. A method of processing a fecal sample, the method comprising:

A) obtaining a pair of portions of a fecal sample from a human subject, the pair of portions comprising a removed portion and a stabilized remaining portion of a fecal sample obtained by the method of claim 82, and

B) testing the removed portion of said fecal sample for the presence of blood.

88. The method of claim 87, wherein said testing comprises testing the removed portion of said fecal sample for a concentration of hemoglobin present in said removed portion.

89. The method of claim 87, wherein testing for the concentration of hemoglobin in said removed portion comprises immunochemical detection of hemoglobin.

90. The method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 5 ng/ml.

91. The method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 10 ng/ml.

92. The method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 20 ng/ml.

93. The method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 50 ng/ml.

94. The method of claim 88, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 200 ng/ml.

95. The method of claim 87, further comprising

C) extracting DNA from the stabilized remaining portion of said fecal sample; and

D) analyzing said DNA for the presence of DNA from a human, wherein the DNA from a human has an epigenetic modification.

96. The method of claim 95, wherein said epigenetic modification comprises aberrant

methylation.

97. The method of claim 95, wherein said aberrant methylation comprises hypermethylation.

98. The method of claim 95, wherein said DNA from a human comprises a gene and/or a promoter region of a gene.

99. The method of claim 98, wherein said gene selected from the group consisting of PHACTR3, NDRG4, FOXE1 , GATA4, GPNMB,TFPI2, SOX17, SYNE1 , LAMA, MMP2, OSMR, SFRP2, and CDO1.

100. The method of claim 96, wherein analyzing said DNA comprises modifying said DNA with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil.

Electronic Patent Application Fee Transmittal

Application Number:	15634607
Filing Date:	27-Jun-2017
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Filer:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	1252	1	640	640
Miscellaneous:				
		Total in USD ($)		**640**

Electronic Acknowledgement Receipt

EFS ID:	42129801
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow
Filer Authorized By:	
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	09-MAR-2021
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Appeal Brief Filed	2021-03-09_35239-304_AppellantsBriefFINAL.pdf	307666 107a1dbe096526d3b353107cea3abbff685b7762	no	24
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30860 24b421fbfa51b77ab86e836a67fb2314d1691910	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	338526		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 06/28/2021

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EXAMINER	
SISSON, BRADLEY L	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
06/28/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@CASIMIRJONES.COM
Genomic_docketing@cardinal-ip.com

Office Action Summary	Application No. 15/634,607	Applicant(s) Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 09 March 2021.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 82-84 and 87-100 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 82-84 and 87-100 is/are rejected.

8) ☑ Claim(s) 82-83 is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Prosecution Reopened after Appeal Brief

1. In view of the appeal brief filed on 09 March 2021, PROSECUTION IS HEREBY REOPENED. New ground of rejection is set forth below.

2. To avoid abandonment of the application, appellant must exercise one of the following two options:

(1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,

(2) initiate a new appeal by filing a notice of appeal under 37 CFR 41.31 followed by an appeal brief under 37 CFR 41.37. The previously paid notice of appeal fee and appeal brief fee can be applied to the new appeal. If, however, the appeal fees set forth in 37 CFR 41.20 have been increased since they were previously paid, then appellant must pay the difference between the increased fees and the amount previously paid.

3. A Supervisory Patent Examiner (SPE) has approved of reopening prosecution by signing below:

/DAVE T NGUYEN/
Supervisory Patent Examiner, Art Unit 1634

Notice of Pre-AIA or AIA Status

4. The present application is being examined under the pre-AIA first to invent provisions.

Claim Objections

5. Claims 82 and 83 are objected to because of the following informalities: In claim 82, there is no conjunctive (and) or disjunctive (or) following step c), and before step d). Similarly,

claim 83 lacks a conjunctive or disjunctive following step i), but before step ii). Appropriate correction is required.

Claim Rejections - 35 USC § 103

6. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

7. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Attention is directed to the decision in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007):

> When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

9. It is further noted that prior art is not limited to the four corners of the documentary prior art being applied. Prior art includes both the specialized understanding of one of ordinary skill in the art, and the common understanding of the layman. It includes "background knowledge possessed by a person having ordinary skill in the art. . . [A] court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR* at 1396.

10. Suggestion, teaching or motivation does not have to be explicit and "may be found in any number of sources, including common knowledge, the prior art as a whole or the nature of the problem itself" *Pfizer, Inc. v. Apotex, Inc.* 480 F.3d 1348, 82 USPQ2d 1321 (Fed. Cir. 2007) citing *Dystar Textilfarben GMBH v. C. H. Patrick Co.*, 464 F.3d 1356 (Fed. Cir. 2006).

Admissions as Prior Art.

11. Attention is directed to MPEP 706.02 [R-07.2015]:

> **III. RELIANCE ON ADMITTED PRIOR ART IN SUPPORT OF REJECTION**
> A statement by an applicant in the specification or made during prosecution identifying the work of another as "prior art" is an admission which can be relied upon for both anticipation and obviousness determinations, regardless of whether the admitted prior art would otherwise qualify as prior art under the statutory categories of 35 U.S.C. 102. *Riverwood Int'l Corp. v. R.A. Jones & Co.*, 324 F.3d 1346, 1354, 66 USPQ2d 1331, 1337 (Fed. Cir. 2003); *Constant v. Advanced Micro-Devices Inc.*, 848 F.2d 1560, 1570, 7 USPQ2d 1057, 1063 (Fed. Cir. 1988). See MPEP § 2129 for discussion on admissions as prior art. Where the admitted prior art anticipates the claim but does not qualify as prior art under any of the paragraphs of 35 U.S.C. 102, the claim may be rejected as being anticipated by the admitted prior art without citing to 35 U.S.C. 102.

12. Attention is directed to MPEP 2129 [R-08.2012], Admissions as Prior Art, which states in part:

> **I. ADMISSIONS BY APPLICANT CONSTITUTE PRIOR ART**
> A statement by an applicant in the specification or made during prosecution identifying the work of another as "prior art" is an admission **>which can be relied upon for both anticipation and obviousness determinations, regardless of whether the admitted prior art would otherwise qualify as prior art under the statutory categories of 35 U.S.C. 102. *Riverwood Int'l Corp. v. R.A. Jones & Co.*, 324 F.3d 1346, 1354, 66 USPQ2d 1331, 1337 (Fed. Cir. 2003); *Constant v. Advanced Micro-Devices Inc.*, 848 F.2d 1560, 1570, 7 USPQ2d 1057, 1063 (Fed. Cir. 1988).

Holding and Rationale

13. Claims 82-83, and 87 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41) in view of US 2008/0124714 A1 (Shuber et al) and US 2006/0216830 A1 (Kikuiri).

14. Kutzner et al., disclose method for the detection of colorectal tumors using a combination of tests, one which looks for a component of blood, and one which is based on nucleic acid analysis, e.g., PCR.

15. A seen therein, the faecal occult blood test was "a standard guaiac test", which is not an immunological test, deemed deficient, and does not seek to determine the amount of hemoglobin present. Kutzner et al., have not been found to teach freezing a portion of the sample (claim 83) or assaying for aberrant methylation, including the aspect of modifying DNA with a bisulfite ion which results in unmethylated cytosine being converted to uracil (claims 95-98 and 100).

16. Shuber et al., teach methods for stabilizing biological samples for nucleic acid analysis. As seen at paragraph [0009], the sample can be that of a patient's fecal sample. Shuber et al., paragraph [0031], teach:

> However, according to the invention, it may be particularly important to preserve sample DNA for purification, especially when looking for early indicia of diseases (e.g., indicia of adenomas or early stage cancers that may be present in less than about 1%, or about 0.1% or less of human genomes isolated from a stool sample). **A common method to insure that DNA remains stable is to freeze stool samples as quickly as possible after collection, or to receive samples in centralized testing labs as quickly as possible**. (Emphasis added)

17. Shuber et al., in Example 2, teach the benefits of treating a stool sample with a nucleic acid stabilization solution. Addition of stabilizer and the freezing step are also disclosed on page 32. And in Example 3, "The Effect of Stabilization Buffers on DNA Integrity and Multiple Mutation Assays", Shuber et al., teach performing nucleic acid analysis on frozen samples where

a stabilizing agent had been used. Such a showing is deemed to meet a limitation of claims 82 and 83.

18. As evidenced above, Shuber et al., teach freezing the sample, be it for immunological testing or analysis of the nucleotide sequence of a gene, and the methylation thereof.

19. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the methods of Kutzner et al., whereby one was detecting for colorectal cancer by using multiple distinct portions from each fecal sample, as shown above. Such methods had been developed to the point that one would have been motivated to obtain a high degree of sensitivity by directing a parallel nucleic acid assay for known, informative genes, whereby one was using different portions of a faecal sample for the immunoassay and the nucleic acid-based assay. See Kultzer et al. It would have also been obvious to said ordinary artisan to have employed means of stabilizing the sample portions for to do so would have a direct an immediate effect of being able to accurately and reproducibly determine the level of hemoglobin present, as well as preserve any nucleic acid present in the cells, and therein enhancing the ability to obtain accurate results, as suggested by Shuber.

20. Claims 87-94 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41) in view of US 2008/0124714 A1 (Shuber et al), as applied to claim 82, and further in view of US 2006/0216830 A1 (Kikuiri) and Yeh (US 2008/0227298 A1).

21. The rejection of claim 82 as being obvious over Kutzner in view of Shuber is applied here as indicated above. Kutzer in view of Shuber does not teach limitations such as delivering

fecal samples and separate portions to a lab, hemoglobin detection with various concentrations that are known in the prior art as indicative of either a threshold level or upper level therefrom.

22. However, Kikuiri, at paragraph [0051], teaches that container with a fecal specimen can be transported/delivered to a medical facility for testing. As stated therein:

> [0051] The feces sampling container 1 containing the fecal suspension 17a is directly transported to a medical facility or the like, and subjected to a quantitative analysis of a specific component included in the fecal suspension 17a. With reference to FIGS. 6 to 8, the structure of an analyzer will be described, by taking an analyzer for measuring a hemoglobin concentration in the fecal suspension 17a as an example. (Emphasis added)

23. As evidenced above, the sample can be tested for concentration of hemoglobin. The above showing is deemed to meet a limitation of claim 84.

24. While Kikuiri teaches detecting hemoglobin in a fecal sample, Kikuiri has not been found to teach the concentration of hemoglobin that can be detected.

25. Yee et al., paragraph [0041], teach setting a 10 ng/ml of hemoglobin in blood as a threshold value. Such a showing is deemed to meet a limitation of claims 87-94.

26. Applicant, at page 2 of the as-filed disclosure, under "BACKGROUND OF THE INVENTION," teaches that various methods, including immunochemical assays for detecting human hemoglobin in fecal sample was known in the art. As stated therein:

> Fecal occult blood tests fall primarily into two categories, tests based on the use of chromogenic chemical reagents such as gum guaiac and immunochemical tests. The chemically based guaiac methods determine the presence of occult blood by the detection of the perioxidase activity of the hemoglobin in the blood present in the faecal sample. They require catalysis of peroxide into oxygen and water, and the subsequent oxidation of a colorless dye (most often into a colored form). However, peroxidase activity is also found in meats and vegetables. In order to produce accurate results, these tests require restriction of the intake of certain foods, drugs, vitamins, and other substances prior to and during the sample collection period. The sensitivity of the most commonly used guaiac FOBT (Hemoccult) is approximately 50%. Despite a specificity of 98%, the positive predictive value for FOBT is low. Methods of detecting occult blood based on porphyrin (heme and protpoporphyrin IX) analysis or immunologic tests using anti-hemoglobin antibodies improve on these results**. Immunochemical tests (FIT or**

> **iFOBT) that use anti- hemoglobin antibodies specific for human blood in extracts from stool do not require dietary restrictions;** however, they are more complicated and more expensive than peroxidase-based tests. (Emphasis added).

27. It would have also been obvious to an ordinary artisan to have employed distinct portions from a subject's fecal sample in testing lab, as exemplified by Kikuiri, to detect multiple signals indicative of colorectal cancer or tumor, e.g., an inclusion of detecting occult blood (hemoglobin) at various desired contractions based on known threshold levels. The ordinary artisan would have been motivated perform multiple testing on different targets present in a fecal sample in order to enhance various degrees of accurate diagnosis of colorectal cancer, as taught and suggested by the combined cited references, especially hemoglobin detections with established thresholds are well established in the prior art as exemplified by Yee.

28. Claims 87, 95-100 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41) in view of US 2008/0124714 A1 (Shuber et al), as applied to claim 87, and further in view of either Kim et al. (BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008) or WO 2008/084219 A1 (Van England et al.).

29. The rejection of claim 87 as being obvious over Kutzner in view of Shuber is applied here as indicated above. Kutzer in view of Shuber does not teach limitations such as specific non-invasive molecular biomarker detection with various epigenetic biomarkers. e.g., hypermethylation level.

30. However, Kim teaches colorectal cancer epigenetic biomarkers are used for the early detection along of colorectal cancer with fecal-occult blood test (FOB). See page 686, Table 1, particularly column 1 bridging column 2 of page 689.

31. Applicant, page 5 of the disclosure, teaches that methods for detection of colorectal carcinoma based upon determining methylation of a number of different genes, including panels of genes, is disclosed in WO 2008/084219 A1 (Van England et al.).

> Van England relates to detection of colorectal cancer based upon determining methylation of a number of different genes, including panels of genes. The elected marker NDRG4 is also taught in Van England et al. (claims 1 and 2).

32. Applicant, at page 21, last paragraph, bridging to page 22 of the as-filed disclosure admits that numerous methods for assessing methylation are known in the art, including methods that comprise polymerase chain reaction (PCR), which is recognized as a form of amplifying DNA. Also admitted to therein is the use of chemical reagents so to modify the DNA prior to amplification was also well known in the art. As admitted to therein:

> **Determination of the methylation status may be achieved through any suitable means. Suitable examples include bisulphite genomic sequencing and/or by methylation specific PCR. Various techniques for assessing methylation status are known in the art and can be used in conjunction with the present invention**: sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR(McMS-PCR), MLPA with or without bisulphite treatment, QAMA (Zeschnigk et al, 2004), MSRE-PCR (Melnikov et al, 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulphite conversion-specific methylation-specific PCR (BS-MSP)(Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence diferences in PCR products of sodium bisulphite - treated DNA), methylation-sensitive single-nucleotide primer extension conformation(MS-SNuPE), methylation- sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulphite restriction analysis (McCOBRA)(Akey et al., 2002), PyroMethA, HeavyMethyl (Cottrell et al. 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques for DNA methylation analysis is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264, Nature Reviews, 2003, Vol.3, 253-266; Oral

> Oncology, 2006, Vol. 42, 5- 13, which references are incorporated herein in their entirety. (Emphasis added)

33. Applicant, at page 24, third paragraph, admits that methods for performing MSP, wherein one employs primer pairs designed to distinguish methylated DNA from unmethylated DNA, was known in the art. As admitted to therein:

> In the MSP approach, DNA may be amplified using primer pairs designed to distinguish methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulphite treatment (**Herman et al., 1996; and WO 97/46705**). For example, bisulphite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. (Emphasis added)

34. The above showing is deemed to meet a limitation of claims 95, 96, and 100.

35. Applicant, at page 26, last paragraph, bridging to page 27, of the as-filed disclosure further asserts:

> Whilst PCR is the preferred nucleic acid amplification technique, other amplification techniques may also be utilised to detect the methylation status of the concerned gene. Such amplification techniques are well known in the art, and include methods such as NASBA (Compton, 1991), 3SR (Fahy et al., 1991) and Transcription Mediated Amplification (TMA). Other suitable amplification methods include the ligase chain reaction (LCR) (Barringer et al, 1990), selective amplification of target polynucleotide sequences (US Patent No. 6,410,276), consensus sequence primed polymerase chain reaction (US Patent No 4,437,975), arbitrarily primed polymerase chain reaction (WO 90/06995), invader technology, strand displacement technology, and nick displacement amplification (WO 2004/067726). This list is not intended to be exhaustive; any nucleic acid amplification technique may be used provided the appropriate nucleic acid product is specifically amplified. Thus, these amplification techniques may be tied in to MSP and/or bisulphite sequencing techniques for example. (Emphasis added)

36. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the methods of Kutzner et al. in view of Shuber, whereby one was detecting hemoglobin via an immunoassay for as shown above. Such methods had been developed to the point that one would have been motivated to obtain a high degree of sensitivity by directing a parallel nucleic acid assay for known, informative genes, such as the

elected NDG4, TFPI2, PHACTR3, SFRP2 and SOX17, whereby one was using different portions of a faecal sample for the immunoassay and the nucleic acid-based assay, e.g., hypermethylation detection assays. It would have also been obvious to said ordinary artisan to have employed means of stabilizing the sample portions for to do so would have a direct an immediate effect of being able to accurately and reproducibly determine the level of hemoglobin present, as well as preserve any nucleic acid present in the cells, and therein enhancing the ability to obtain accurate results.

37. In view of the teaching of the combined cited art such as Kutzner and Kim, which teaches a desire for accurate detection of early colorectal cancer with usages of various known molecular DNA biomarkers in addition with the FOBT, one of ordinary skilled artisans would have been motivated have employed admittedly known methods so to detect not only occult blood (hemoglobin) but known and useful gene targets, and to also determine whether the target sequences such as NDRG4 also demonstrate hypermethylation, be it in a gene of interest or a promoter of same.

38. In view of the well-developed state of the art and significant interest in being able to detect cancer at an early stage via both immunochemistry and nucleic acid analysis, said ordinary artisan would have been both well motivated and would have had a most reasonable expectation of success.

Conclusion

39. Objections and/or rejections which appeared in the prior Office action and which have not been repeated hereinabove have been withdrawn.

40. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Bradley L. Sisson whose telephone number is (571)272-0751. The examiner can normally be reached on Monday to Thursday, from 6:30 AM to 5 PM.

41. Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

42. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

43. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Bradley L. Sisson/

Primary Examiner, Art Unit 1634

Notice of References Cited	Application/Control No. 15/634,607	Applicant(s)/Patent Under Reexamination Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20060216830-A1	09-2006	Kikuiri; Hideki	B01L3/5082	436/165
*	B	US-20080227208-A1	09-2008	Yee et al.	G01N33/72	436/66
	C					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
*	N	WO-2004083399-A2	09-2004	WO	Goggins et al.	C12Q1/6886
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Kutzner et al., "Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", Cancer Letters", 229 (2005), pp. 33-41. (Year: 2005)
	V	Kim et al., "Noninvasive molecular biomarkers for the detection of colorectal cancer", BMB reports, Volume 41, Issue 10, pp 685-692, September 2008
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021				
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
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	94	÷	✓	✓	✓	✓				

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
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	100	÷	✓	✓	✓	✓				

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.
EAST	06/16/2021	B. L. S.

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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Non-invasive detection of colorectal tumours by the combined application of molecular diagnosis and the faecal occult blood test

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Abstract

The treatment of early-stage tumours decreases the overall mortality of colorectal tumour patients. In this retrospective study we determined the sensitivity and the specificity of the faecal occult blood test (FOBT) and the molecular diagnosis (MD). We analysed 57 stool samples from patients with colorectal carcinomas for the presence of occult blood using a standard FOBT and for alterations in the three different tumour relevant markers APC, BAT26 and L-DNA. Stool samples from 44 control donors were analysed to determine the specificity of the applied methods. Twenty-nine (51%; 95% confidence interval (CI): 38–63%) stool samples of the cancer patients gave positive FOBT results. Thirty-seven (65%; CI: 52–76%) samples showed alterations in at least one DNA marker. Sixteen (28%) samples were positive only in the FOBT, and 24 (42%) samples showed a positive result exclusively in MD. The combined application of both methods resulted in a sensitivity of 93% (CI: 83–97%) and an overall specificity of 89% (CI: 76–95%). The combined application of FOBT and MD resulted in an overall sensitivity, which could not be achieved by any of the methods alone and which is in the range of invasive diagnostic methods.
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Keywords: Non-invasive colorectal cancer diagnosis; Faecal DNA analysis; Molecular diagnosis; Faecal occult blood test

1. Introduction

The diagnosis and the therapy of early-stage tumours have the potential to decrease morbidity and mortality of colorectal cancer patients. Invasive methods like sigmoidoscopy, colonoscopy and

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roentgenography are valuable tools for the specific and sensitive detection and the exact localization of a colorectal tumour (reviewed in [1]). These techniques are unsuitable for broad screening programs mainly because of their low general acceptance [2]. The most common screening method is the faecal occult blood test (FOBT). Randomised trials revealed the clinical effectiveness in reduction of mortality through the implementation of the FOBT [3–5].

Sidransky et al. introduced the analysis of faecal DNA for tumour specific alterations as a novel non-invasive diagnostic strategy [6]. Until now several studies have proven the usefulness and the feasibility of faecal DNA analysis (reviewed in [7]). Recent multi-target studies achieved sensitivities between 62 and 91% and specificities up to 98%, respectively [8–10]. A few studies compared the outcomes of the molecular diagnosis (MD), which is the detection of tumour relevant alterations in faecal DNA, with these of the FOBT. The finding that 9 out of 11 asymptomatic adenoma patients were identified by the analysis of five different DNA markers, whereas none was detected by FOBT, indicated a higher diagnostic sensitivity of the MD [9]. The data of a large prospective study, which is close to completion, will allow assessing the two methods and their combined application (annual meetings of the American College of Gastroenterology (ACG), Baltimore 2003 and of the American Gastroenterology Association (AGA), New Orleans 2004). We intended to judge in this retrospective study whether the analysis of three faecal DNA markers has the potential to complement or even to replace the FOBT and whether the combined application of the two methods might increase the overall diagnostic reliability.

2. Materials and methods

2.1. Study population and sample collection

All patients and control donors provided their informed oral and written consent to provide samples. This study was approved by the responsible ethical committees at the Klinik für Chirurgie Marienhospital (Herne) and at the Berufsgenossenschaftliche Kliniken Bergmannsheil (Bochum). None of the patients and none of the control donors had familial adenomatous polyposis coli or hereditary non-polyposis colorectal cancer. Patients were diagnosed with colorectal tumours at the Berufsgenossenschaftliche Kliniken Bergmannsheil, the Marienhospital Herne or the Katholisches Krankenhaus Dortmund-West between 1998 and 2003. All tumours were diagnosed and located by imaging methods like colonoscopy and roentgenography. At least seven days after colonoscopy and between 2 and 4 days before surgical removal of the tumour 2 samples were taken from regular stools, immediately frozen at −20 °C and transferred to −80 °C within 24 h. The surgically removed tumours were classified according to the AJCC/TNM system (American Joint Committee on Cancer (AJCC) stages I through IV; Table 1) [11]. Because of personnel alternations in the clinical departments in the course of the study, we could not reproduce the staging information for 12 tumours, the age and the gender of 8 patients and the location for 3 tumours at the time of the analysis of the faecal samples. Control stool samples were from 24 female and 20 male donors, who volunteered to participate. The age group of control donors was 24–78 years (mean age 49). Control donors did not show any symptoms or signs of colorectal diseases as determined in personal interviews.

2.2. FOBT

Two stool samples from each cancer patient and one sample from each control donor were analysed for the presence of occult blood by a standard guaiac test using commercial reagents and standard protocols (Care Diagnostica). Each FOBT was performed twice. A blue colour reaction within 120 s after the addition of the hydrogen peroxide solution was rated as a positive result. In case the double FOBT analysis of one stool sample or of two stool samples from one patient gave discrepant results, the sample was rated positive.

2.3. MD

Faecal DNA from all samples was purified using a method, which was optimised for faecal specimen [12]. An essential step of this method is the matrix-based elimination of PCR inhibiting and DNA damaging compounds (Stool Mini Kit, Qiagen).

Table 1
Patient and tumor data of all samples and results obtained by the analysis of stool samples from cancer patients by FOBT and by MD

Patient	Gender/age	AJCC/TNM	FOBT	MD positive markers
79	M/70	n.a.*	neg	neg
80	M/58	II/T3N0MxG2	pos	APC
83	M/67	II/T3N0MxG2	neg	APC, L-DNA
84	M/85	II/T3N0MxG2	neg	BAT26
87	n.a.	n.a.	neg	APC
88	F/76	II/T3N0MxG2	pos	neg
89	n.a.	n.a.	neg	L-DNA
90	F/62	I/T2N0MxG2	neg	L-DNA
92	F/53	n.a.	pos	neg
93	n.a.	n.a.	pos	APC
95	F/68	I/T2N0MxG2	pos	APC
96	n.a.	n.a.	neg	APC, L-DNA
121	M/51	I/T2N0MxG2	neg	L-DNA
136	M/61	I/T2N0MxG2	neg	APC
138	M/65	I/T2N0MxG2	pos	neg
139	M/74	II/T3N0MxG2	neg	neg
140	F/73	II/T3N0MxG2	pos	neg
141	n.a.	n.a.	pos	APC
172	F/75	II/T3N0MxG2	neg	APC
173	M/73	II/T3N0MxG2	neg	APC
174	F/79	IV/T3N2M1hepG2	pos	APC, L-DNA
175	F/75	II/T3N0MxG3	pos	neg
176	M/63	IV/T3N1M1hepG3	neg	APC
179	M/68	III/T3N1MxG2	neg	APC
180	F/65	II/T4N0MxG2	neg	APC
181	F/92	III/T4N1M0G2	pos	neg
182	M/74	I/T2N0MxG2	neg	APC
183	M/60	II/T3N0MxG2	neg	L-DNA
184	M/65	IV/T3N2M1hepG2	neg	L-DNA
185	M/75	I/T2N0MxG2	pos	BAT26
186	M/47	III/T3N1MxG2	neg	L-DNA
187	M/73	II/T3N0MxG2	pos	neg
188	M/65	IV/T3N1M1hepG2	neg	neg
189	M/76	III/T3N2MxG2	neg	APC, L-DNA
190	M/69	I/T2N0MxG2	pos	BAT26, L-DNA
191	M/76	III/T3N1MxG2	pos	L-DNA
192	M/89	II/T3N0MxG2	pos	L-DNA
193	F/64	I/T2N0MxG2	neg	BAT26, L-DNA
251	M/55	IV/T3N2M1G2	pos	L-DNA
252	M/74	II/T3N0MxG2	pos	neg
253	F/83	III/T3N1MxG2	neg	L-DNA
254	M/66	II/T4N0MxG2	pos	BAT26, L-DNA
255	M/62	III/T3N2MxG2	pos	neg
257	n.a.	n.a.	neg	APC
274	F/70	I/T2N0MxG3	pos	neg
275	n.a.	n.a.	pos	APC, L-DNA
276	n.a.	n.a.	neg	L-DNA
278	M/86	n.a.	neg	L-DNA
283	M/82	III/T3N1MxG2	pos	APC, L-DNA
284	F/72	n.a.	neg	L-DNA
285	M/81	II/T3NxMxG2	neg	neg
286	M/64	IV/T3NxM1hepG2	pos	neg

(*continued on next page*)

Table 1 (*continued*)

Patient	Gender/age	AJCC/TNM	FOBT	MD positive markers
287	M/92	III/T3N1MxG3	pos	neg
289	M/71	II/T4NxMxG3	pos	neg
290	M/66	IV/T4N0M1	pos	neg
292	M/80	I/T1N0MxG2R0	pos	neg
294	F/83	III/T3N2MxG2	pos	neg

n.a., data not available

The quality of the purified DNA was validated by agarose gel electrophoresis. One tenth of the DNA obtained from one stool sample was used in a capturing step to enrich the human DNA from the DNA of non-human origin [13]. The overall yield of the capturing could be increased by the restriction digestion of the DNA with the enzyme DraI. To fish the APC gene out of the pool of genomic DNA, 10 pmol biotin labelled primer APC1 (5′-TTAAAA-TATGCCACAGATATTCCT TCA TCACAGAAA-CAGT-3′) was added to 100 μl DraI digested stool DNA. After denaturing and re-annealing 10 μl streptavidin coupled magnetic beads (Dynal) were added and the mixture was incubated over night at room temperature. After washing, the beads coupled DNA was completely used as template in a first PCR. The APC gene region from codon 1069 through codon 1984 including the mutation cluster region was analysed by PCR and by PTT of two overlapping fragments. The detailed experimental conditions of the following PTT have been published [13] (Hauss and Müller, The protein truncation test in mutation detection and molecular diagnosis, in: Methods in Molecular Biology, edited by Guido Grandi, The Humana Press, in press). Briefly, the primers of the first PCR coded the HA-tag, and the primers of the second PCR coded the Kozak motif and the T7 promoter sequence, which are necessary for the following PTT. The first PCR was carried out at the following conditions: 95 °C (5 min)//[94 °C (30 s)/56 °C (30 s)/72 °C (90 s)] 20×//[94 °C (30 s)/58 °C (30 s)/72 °C (90 s)] 20×//72 °C (10 min) and under the use of the following primers: APC fragment I: 5′-CGCCATGTACCCCTACGACGTGCCCGACTAC GCCTTCCAACCACATTTTGGACAGCAG-3′ and 5′-CCGTCATTTTTCTGCCTCTTTCTCTTGGTT-3′; APC fragment II: 5′-CGCCATGTACCCCTAC-GACGTGCCCGACTACGCCGATGTGGAATTAA-GAATAATGCCT-3′ and 5′-CCGTCATTTTCTT-TATTGTTGTTTTCTTGGTC-3′. One fifth of the volume of the first PCR was used in a second PCR at the conditions: 95 °C (5 min)//[94 °C (30 s)/60 °C (30 s)/72 °C (90 s)] 40×//72 °C (10 min) and using the following primers, which anneal within the products of the first PCR: APC fragment I: 5′-ATCCTAATACGACTCACTATAGGGAGCCAC-CATGTACCCCTACGACGTG-3′ and 5′-CCGTCA-TTTTTCTGCCTCTTTCTCTTGGTT-3′; APC frag-ment II: 5′-ATCCTAATACGACTCACTATAGGG-AGCCACCATGTACCCCTACGACGTG-3′ and 5′-CCGTCATTTTCTTTATTGTTGTTTTCTTGGTC-3′. Three of the control samples gave no reproducible results in the PCR reactions. All other faecal DNA samples from control donors and all samples from cancer patients gave robust PCR products. DNA purified from two stool samples from each cancer patient and from one sample from each control donor was analysed for the presence of alterations in 3 different markers. Each positive result was confirmed in a repeated analysis. All samples showing signals, which were different to the wild-type controls, were further examined. For this the PCR fragments were cloned in a T/A cloning vector and the cloned gene fragments were sequenced by conventional methods. The analyses of all positive samples were repeated by starting with the purified DNA in order to limit the number of false positive results. The analysis of alterations in the microsatellite marker BAT26 and the detection of the relative amount of non-apoptotic L-DNA were performed according to published protocols [9,14]. All results are based on the analysis of at least two independently amplified PCR products. The primer sequences for L-DNA analysis were kindly provided by A.P. Shuber and D.A. Ahlquist. In case of discrepant MD results between the two different samples from one patient, DNA was extracted from a new faecal aliquot of the same patient and the analysis was repeated.

Table 2
Localization of tumours relative to the splenic flexure of the colon and number of the corresponding stool samples diagnosed as positive by FOBT or by MD

Site	*N*	FOBT	MD	Combined use
Proximal	27	16	14	25
Distal	27	11	21	26
n.a.	3	2	2	2
Total	57	29	37	53

N, number of samples; n.a., at the time of analysis the information of the tumour localization was not available.

2.4. Statistical evaluation

Ninety-five percent confidence intervals were determined based on the exact binomial distribution. The Fisher exact test was used for comparison of the test results between patients with different tumour stages. To compare the single application of FOBT or MD with the combined application of both methods the McNemar's test was used.

3. Results

The aim of this study was to determine the sensitivity and the specificity of the two non-invasive diagnostic methods, FOBT and MD, which is the analysis of faecal DNA for alterations in the three different markers APC, BAT26 and L-DNA. We analysed stool samples from 57 colorectal cancer patients and from 44 control donors. Twenty-seven tumours were located proximal and 27 tumours were located distal to the splenic flexure of the colon (Table 2). Twenty-eight patients had tumours, which did not spread to proximal or distant lymph nodes (stage I or II) at the time of staging diagnosis. Lymphatic spread was identified in 17 patients (stage III or IV) (Tables 1 and 3).

Twenty-nine stool samples (51%; 95% confidence interval (CI): 38–63%) showed a positive FOBT test (FOBT pos). Thirty-seven faecal DNA samples (65%; CI: 52–76%) were MD positive (MD pos) demonstrating an alteration in at least one of the three tested DNA markers (Tables 1 and 3). The MD positives include nineteen samples (33%) with truncated APC protein fragments in the PTT analysis (Fig. 1). We sequenced these positive samples and found mutations, which result in stop codons between codons 1254 and 1472 (Table 4). The sizes of the predicted translation products of PCR fragments carrying these stop codons correlate with the approximate sizes of the translation products, which were detected in the PTT (Fig. 1). The number of APC mutations detected in the samples was in the range of the numbers

Table 3
Summarized results of the analysis of the stool samples from cancer patients and sensitivity of MD and FOBT

AJCC stage	n.a.	I	II	III	IV	Total
N	12	11	17	10	7	57
Mean age (range)	70.3 (53–86)	67.2 (51–80)	72.7 (47–92)	76.1 (47–92)	65.3 (55–79)	70.9 (47–92)
Gender (male/female)	2/2	7/4	12/5	7/3	6/1	34/15
n.a.	8	0	0	0	0	8
FOBT pos	4	6	9	6	4	29 (51%; 38–63%)
MD pos	10	8	9	6	4	37 (65%; 52–76%)
APC	6	3	5	3	2	19 (33%)
BAT26	0	3	2	0	0	5 (9%)
L-DNA	6	4	4	5	3	22 (39%)
MD pos or FOBT pos	11	11	15	10	6	53 (93%; 83–97%)
MD(APC or L-DNA) pos or FOBT pos	11	11	14	10	6	52 (91%; 81–96%)
MD pos and FOBT neg	7	5	6	4	2	24 (42%; 30–55%)
FOBT pos and MD neg	1	3	6	4	2	16 (28%; 18–41%)

The results, the relative sensitivities and the corresponding 95% confidence intervals are given for the FOBT, the MD and the combined application of the two methods. A sample was rated MD positive (MD pos), when one molecular marker was tested positive in the PTT, in the BAT26 or in the L-DNA analysis. n.a., AJCC staging of the corresponding tumours or age and gender were not available at the time of analysis.

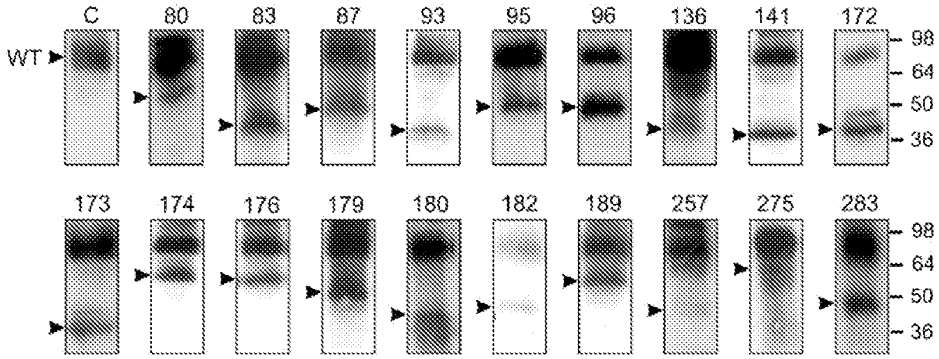


Fig. 1. Positive PTT results of the APC gene analysis. Arrowheads point to bands, which represent the wild-type full-length protein (WT) or smaller proteins, which are truncated due to mutations. On the right side the sizes of marker fragments are indicated in kilodaltons. C, non-mutated control.

described in other studies [9,15]. Ahlquist et al. (2000) detected APC mutations in 24% faecal DNA samples of cancer patients. Traverso et al. (2002) found APC mutations in 63% DNA samples of patients with T3N0M0 tumours. We detected BAT26 patterns different to the wild-type control in 5 (9%) samples (Fig. 2). Twenty-two (39%) samples showed high levels of L-DNA amplification products (Fig. 3). Thirty-five stool samples (61% of all samples or 95% of MD positive samples) showed alterations in the APC gene or in the relative L-DNA level. This high number reflects the high proportion of colorectal tumours, which harbour an APC gene mutation and a dysfunction in the regulation of apoptosis (Tables 1 and 3).

Forty-two percent of the samples (CI: 30–55%) were positive in at least one of the MD markers, but gave negative FOBT results. Twenty-eight percent of the samples (CI: 18–41%) were positive exclusively in the FOBT, but not in the molecular test. When the results of both methods were combined, 53 (93%; CI: 83–97%) samples were positive in FOBT or in molecular testing. The combined application of the two methods together leads to a significantly higher sensitivity than the application of any of the methods alone ($P<0.01$). When FOBT was combined with a limited MD, which is the analysis of the two molecular markers APC and L-DNA, a detection rate of 91% (CI: 81–96%) was achieved. The sensitivity of this combined application is higher than the sensitivity of the analysis by limited MD or by FOBT alone ($P<0.01$).

In one control sample we found occult blood by FOBT resulting in an overall FOBT specificity of 98% (CI: 88–100%) (Table 5). In another control sample we detected a truncated APC protein fragment by PTT. The following sequence analysis revealed a C to T transition at nucleotide position 4117 leading to

Table 4
Sequence alterations in APC detected by sequencing of MD positive samples

Sample	Mutation at sequence position	Resulting stop at codon
80	C4278del	1472
83	C4117T	1367
87	G4153T	1379
93	G4075T	1353
95	C4366T	1450
96	C4366T	1450
136	G3943T	1309
141	A3835T	1273
172	G3943T	1309
173	C3777del	1254
174	4409–4412del	1472
176	A4330del	1472
179	G4152^G4153insA	1414
180	C4117T	1367
182	C4117T	1367
189	T4310^G4311insGCCACCAA	1472
257	C4117T	1367
275	A4382del	1472
283	C4055A	1346

Nucleotide changes, the nucleotide sequence positions and the resulting stop codons are listed for the detected APC mutations. All faecal DNA samples were screened by PTT for alterations in APC. Positive samples were sequenced.

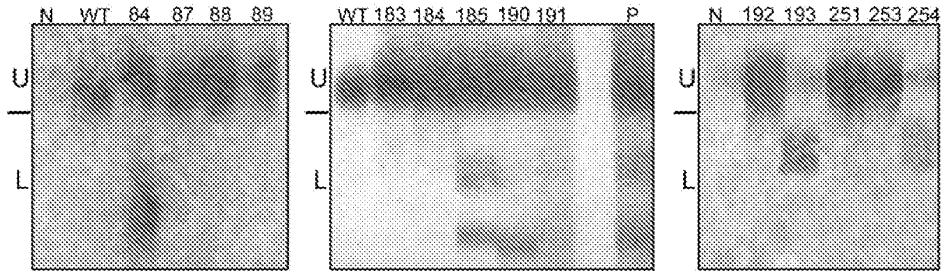


Fig. 2. Polyacrylamide gels for the analysis of alterations in the BAT26 microsatellite patterns. All BAT26 positive samples and 9 representing negative samples are shown. The upper (U) region of the gel contains PCR products representing the wild-type full-length products. Bands in the lower (L) regions of the gels indicate deletions in the BAT26 microsatellite. N, negative control without any DNA template; WT, wild-type control from normal tissue; P, positive control from tumour tissue containing the mutant BAT26.

a stop signal in the corresponding codon 1367 (not shown). Both control donors showed no signs of malignancies or polyps according to colonoscopy analysis. Three samples from the control group did not give reproducible PCR results. In the final statistical calculation of the overall specificity these control samples were rated positive (Table 5). Thus the specificity of MD was 91% (CI: 79–96%), and the overall specificity of the combined application of FOBT and MD was 89% (CI: 76–95%).

4. Discussion

Tumour specific mutations in faecal DNA are supposed to be sensitive and specific markers for the non-invasive diagnosis of colorectal cancer (reviewed in [7,16]). In this study the sensitivity of the retrospective diagnosis of colorectal carcinoma by the analysis of the three different molecular markers APC, BAT26 and L-DNA was 65%. The sensitivities described by others for the analysis of five different markers reach from 64 to 90% [9,10]. The differences between the sensitivities might be due to variations in sample quality, in the applied analytical methods and in the laboratory-specific handling of the samples. The high potential of the MD became obvious, when we compared the MD outcomes with those of the most common non-invasive screening test, the FOBT. The MD showed a 14% higher detection rate than the FOBT, and 42% of all samples were negative in the FOBT, but still detectable by MD. Nevertheless, our data also confirm the value of the FOBT. Transferring the data to a putative random non-invasive screen 28 out of 100 cancer patients would be detected only by FOBT but not by MD. The sensitivity of MD can be even further increased up to 93% by the additional application of FOBT. While the combination of the FOBT and MD leads to a significant increase in sensitivity, it leads only to a marginal decrease in specificity (Table 5).

There was no significant correlation of the FOBT or MD results with the gender, the age or the tumour stages. This finding is in line with the results of others [9]. Furthermore, we did not find a significant correlation of the positive markers with the location

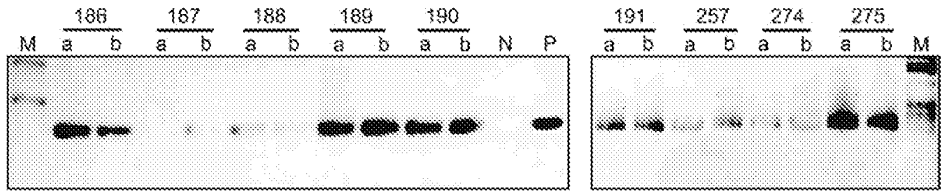


Fig. 3. Agarose gels for the analysis of the amplification of L-DNA. The two gels show the analysis of PCR of KRAS-L-DNA from 9 representing stool DNA samples, which were amplified in duplicates (a, b). M, Marker; N, negative control without any DNA; P, positive control.

Table 5
The numbers of positive samples and Summary of results

	Cancer patients	Control donors	Sensitivity (%)	Specificity (%)
FOBT pos	29	1	51 (38–63)	98 (88–100)
MD pos	37	4	65 (52–76)	91 (79–96)
MD pos or FOBT pos	53	5	93 (83–97)	89 (76–95)
MD(APC or L-DNA) pos or FOBT pos	52	5	91 (81–96)	89 (76–95)

The relative sensitivities, specificities and the corresponding 95% confidence intervals of the MD, the FOBT and of the combined application of the two methods are given. The MD positive samples of the control donors include three samples, which could not be analysed.

of the tumours (Table 2). The findings of others, who described indications for a correlation between tumour location and BAT26 and L-DNA markers, have to be confirmed by the analysis of a high number of samples [9].

In order to determine the specificity we analysed the samples of randomly chosen control donors, who showed no obvious symptoms of colorectal diseases. This strategy might have led to an overestimated specificity in comparison to an authentic screening, which includes both non-symptomatic and symptomatic cancer patients and healthy donors. Because the analysed MD markers APC and BAT26 correlate directly to tumour development and do not necessarily correlate with the onset of symptoms, the analysis of these two markers should not distort the overall specificity. We are still aware that the FOBT outcomes might have led to the overestimation of the overall specificity. A subsequent study, which will include samples from non-symptomatic as well as samples from symptomatic individuals, will clarify this aspect.

This is one of the first studies, which evaluate and directly compare the potential of the two non-invasive methods MD and FOBT in the diagnosis of colorectal carcinomas. The combined application of the two methods together led to a significantly higher sensitivity than the application of any of the methods alone. Regarding the low cost and the little technical expense of the FOBT, we suggest that MD and FOBT should complement rather than replace each other. The sensitivity of the combined application of MD and FOBT is in the range reported for invasive methods. This leads to the question whether both methods should be used together in broad diagnostic screening programs. We are aware that our retrospective data do not allow any conclusion on the potential of this approach in diagnostic screening. Prospective comparative studies, which include a very high number of individuals, have to prove the value of the combined application of MD and FOBT. Preliminary results of a large study were presented in abstract form (ACG and AGA annual meetings). The final results of this study will allow further conclusions about the effectiveness of the two methods and their combined application.

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References

[1] C.J. Kahi, D.K. Rex, Current and future trends in colorectal cancer screening, Cancer Metastasis Rev. 23 (2004) 137–144.
[2] A.L. Frazier, G.A. Colditz, C.S. Fuchs, K.M. Kuntz, Cost-effectiveness of screening for colorectal cancer in the general population, J. Am. Med Assoc. 284 (2000) 1954–1961.
[3] J.S. Mandel, J.H. Bond, T.R. Church, D.C. Snover, G.M. Bradley, L.M. Schuman, F. Ederer, Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study, N. Engl. J. Med. 328 (1993) 1365–1371.
[4] J.D. Hardcastle, J.O. Chamberlain, M.H. Robinson, S.M. Moss, S.S. Amar, T.W. Balfour, et al., Randomised controlled trial of faecal-occult-blood screening for colorectal cancer, Lancet 348 (1996) 1472–1477.

[5] O. Kronborg, C. Fenger, J. Olsen, O.D. Jorgensen, O. Sondergaard, Randomised study of screening for colorectal cancer with faecal-occult- blood test, Lancet 348 (1996) 1467–1471.
[6] D. Sidransky, T. Tokino, S.R. Hamilton, K.W. Kinzler, B. Levin, P. Frost, B. Vogelstein, Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors, Science 256 (1992) 102–105.
[7] K.S. Tagore, T.R. Levin, M.J. Lawson, The evolution to stool DNA testing for colorectal cancer, Aliment. Pharmacol. Ther. 19 (2004) 1225–1233.
[8] D. Calistri, C. Rengucci, R. Bocchini, L. Saragoni, W. Zoli, D. Amadori, Fecal multiple molecular tests to detect colorectal cancer in stool, Clin. Gastroenterol. Hepatol. 1 (2003) 377–383.
[9] D.A. Ahlquist, J.E. Skoletsky, K.A. Boynton, J.J. Harrington, D.W. Mahoney, W.E. Pierceall, et al., Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel, Gastroenterology 119 (2000) 1219–1227.
[10] K.S. Tagore, M.J. Lawson, J.A. Yucaitis, R. Gage, T. Orr, A.P. Shuber, M.E. Ross, Sensitivity and specificity of a stool DNA multitarget assay panel for the detection of advanced colorectal neoplasia, Clin. Colorectal. Cancer 3 (2003) 47–53.
[11] F.L. Greene, D.L. Page, I.D. Fleming, A. Fritz, C.M. Balch, D.G. Haller, M. Morrow,, sixth ed. AJCC Cancer Staging Manual, XIV, 2002.
[12] R. Deuter, S. Pietsch, S. Hertel, O. Muller, A method for preparation of fecal DNA suitable for PCR, Nucleic Acids Res. 23 (1995) 3800–3801.
[13] S. Kahmann, P. Herter, C. Kuhnen, K.M. Muller, G. Muhr, D. Martin, et al., A non-radioactive protein truncation test for the sensitive detection of all stop and frameshift mutations, Hum. Mutat. 19 (2002) 165–172.
[14] G. Traverso, A. Shuber, L. Olsson, B. Levin, C. Johnson, S.R. Hamilton, et al., Detection of proximal colorectal cancers through analysis of faecal DNA, Lancet 359 (2002) 403–404.
[15] G. Traverso, A. Shuber, B. Levin, C. Johnson, L. Olsson, D.J. Schoetz, et al., Detection of APC mutations in fecal DNA from patients with colorectal tumors, N. Engl. J. Med. 346 (2002) 311–320.
[16] D.A. Ahlquist, A.P. Shuber, Stool screening for colorectal cancer: evolution from occult blood to molecular markers, Clin. Chim. Acta 315 (2002) 157–168.

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Mini Review

Noninvasive molecular biomarkers for the detection of colorectal cancer

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Colorectal cancer (CRC) is the third most common malignancy in the world. Because CRC develops slowly from removable precancerous lesions, detection of the disease at an early stage during regular health examinations can reduce both the incidence and mortality of the disease. Although sigmoidoscopy offers significant improvements in the detection rate of CRC, its diagnostic value is limited by its high costs and inconvenience. Therefore, there is a compelling need for the identification of noninvasive biomarkers that can enable earlier detection of CRC. Accordingly, many validation studies have been conducted to evaluate genetic, epigenetic or protein markers that can be detected in the stool or in serum. Currently, the fecal-occult blood test is the most widely used method of screening for CRC. However, advances in genomics and proteomics combined with developments in other relevant fields will lead to the discovery of novel non invasive biomarkers whose usefulness will be tested in larger validation studies. Here, noninvasive molecular biomarkers that are currently used in clinical settings and have the potential for use as CRC biomarkers are discussed. [BMB reports 2008; 41(10): 685-692]

Introduction

Colorectal cancer (CRC) is the third most common malignancy in the world. In addition, there are approximately 1,000,000 new cases of CRC and 500,000 deaths associated with CRC each year. Indeed, CRC represents one of the primary causes of cancer deaths in Europe and the USA (1). In Korea, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing (2). CRC is believed to develop slowly via a progressive accumulation of genetic mutations; therefore, the risk of recurrence and subsequent death due to CRC is closely related to the stage of the disease at the time of primary diagnosis. Recent studies have shown that shifting the detection of the disease to an earlier stage via mass screening and intervening at this stage can reduce the risk of death from CRC (3, 4). These findings strongly demonstrate the clinical need for biomarkers for early detection of CRC.

Biomarkers are substances that are used as indicators of a biological state. Accordingly, biomarkers have characteristics that enable them to be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (5). One of the key requirements of a test for CRC is that it must allow detection of the disease at earlier stages so that the disease can be cured effectively. Accordingly, such tests should have high sensitivity and specificity while producing a low number of false-negative and false-positive results to prevent subjecting healthy individuals to unnecessary colonoscopies. Sigmoidoscopy offers significant improvements in detection rates for CRC. However, the diagnostic value of sigmoidoscopy is limited with regards to costs, risks, and inconvenience (6, 7). Noninvasive biomarkers can be analyzed relatively easily and economically; therefore, they have the potential to greatly enhance screening acceptance. Several noninvasive tests for CRC detection are available, of which the fecal-occult blooding test (FOBT) is the most commonly used (8-10). However, this method lacks sensitivity as well as specificity for screening an average risk population. As a result, new cancer biomarkers that will further enhance the detection of the disease and trigger follow-up colonoscopies when necessary must be developed. In addition to such screening biomarkers, prognostic markers that predict the likely course of the cancer, stratification markers that predict the likely response to a drug prior to beginning treatment, and efficacy markers that monitor the efficacy of drug treatment may eventually reduce the mortality rate of CRC.

Recent advances in genomics and proteomics have contributed to our understanding of pathways that control the growth, differentiation, and death of cells. Genomic techniques such as DNA microarray analysis and proteomic methods such as 2-dimensional electrophoresis and mass spectrometry are now commonly used to evaluate the expression profiles of genes and proteins in cells, tissues, and bodily fluids (11, 12).

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Exact Sciences Ex. 2003, Page 586 of 1394

Identification of genes or proteins that are characteristic of the development of cancer can potentially uncover biomarkers that will aid in the diagnosis of CRCs. This review will focus on potential biomarkers that have recently been discovered and may be used in the future to detect CRC using non-invasively-collected samples as well as biomarkers that are currently being used in clinical settings (Table 1).

Fecal markers

Fecal hemoglobin

Stool-based screening for CRC is simple, inexpensive and the least invasive method of screening available (13). FOBT, which is the most widely used screening modality for CRC, detects hemoglobin enzymatically or immunologically (14). Enzymatic FOBT measures the peroxidase-like activity of hemoglobin that originates from any source; therefore, it is susceptible to both colorectal and upper gastrointestinal bleeding. In addition, the ingestion of certain foods (red meats, fruits and vegetables) and medicines (non-steroidal anti-inflammatory drugs) can also yield false-positive results. Immunological FOBT uses antibodies that specifically detect human hemoglobin; therefore, it is not impacted by plant peroxidase in the diet. An important limitation of the FOBT is its relatively poor sensitivity at detecting early-stage lesions. In addition, FOBT shows low sensitivity for the detection of both adenomas (~10%) and CRCs (40-85%). Finally, large randomized clinical trials have shown that FOBT is not very reliable and that they reduce cancer mortality by only 30% (3, 15).

Genes and epigenetic markers

Colonocytes, which are shed into the fecal stream, provide informative material that can be used to detect genes and epigenetic markers in feces (16). Unlike fecal blood, which is shed only intermittently, it is believed that colonocytes are shed continuously. Furthermore, the shedding of colonocytes from CRC occurs more frequently than from normal colonic epithelium. Fecal colonocytes are assessed by analyzing DNA mutations for targets such as K-*ras*, *p53*, and adenomatous polyposis coli (*APC*), by analyzing epigenetic markers such as microsatellite instability (MSI), or by measuring unfragmented long-form DNA (L-DNA).

K-*ras*, which encodes a Ras family protein, functions as a guanine nucleotide binding protein that is involved in a signal transduction pathway that includes the phosphatidylinositol-3-kinase and serine/threonine protein kinase B pathways (17). K-*ras* mutations have been found in 40-50% of sporadic colon cancers and adenomas (18). In addition, studies have demonstrated that K-*ras* mutations are present in aberrant crypt foci (a suspected pre-cancerous lesion) in 13-95% of all CRC cases (19-21), which suggests that K-*ras* mutations may be an important early event in tumorogenesis.

p53 encodes a tumor suppressor protein that regulates the expression of genes involved in apoptosis, angiogenesis, the cell cycle and maintenance of the genome (22). Approximately 50% of all human cancers contain mutated *p53* genes, and 30-60% of

Table 1. Noninvasive molecular biomarkers for the detection of CRC

Analyte	Subject	Type	Status
Fecal hemoglobin	Stool	Protein	IU
K-*ras*	Stool	DNA	CV
APC	Stool	DNA	CV
L-DNA	Stool	DNA	CV
p53	Stool	DNA	CV
CEA	Serum	Protein	IU
CA19.9	Serum	Carbohydrate	IU
TIMP-1	Serum	Protein	CV
Spondin-2, DcR3, Trail-R2, Reg IV, MIC 1	Serum	Protein	PD
PSME3	Serum	Protein	PD
NNMT	Serum	Protein	PD
CRMP-2	Serum	Protein	PD
SELDI (apolipoprotein C1, C3a-desArg, alpha1-antitrypsin, transferrin)	Serum	Protein	PD
HNP 1-3	Serum	Protein	PD
MIF	Serum	Protein	PD
M-CSF	Serum	Protein	PD
M2-PK	Serum	Protein	PD
Prolactin	Serum	Protein	PD
Septin 9	Plasma	DNA	PD
Five-gene panel (*CDA, BANK1, BCNP1, MS4A1, MGC20553*)	WBC	DNA	PD
CCSA-2, -3 -4	Serum	Protein	PD
MMP-9, -7	Serum	Protein	PD
Laminin	Serum	Protein	PD

IU, in use; CV, clinical validation; PD, preclinical development

Exact Sciences Ex. 2003, Page 587 of 1394

CRCs have demonstrated mutations in the gene that vary based on the stage, grade and location of the cancer (23) These mutations appear to be formed relatively late in the genesis of CRC, and altered *p53* has only a modest impact on outcome. Therefore, the relatively low mutation rate of *p53* in early-stage tumors limits its sole use in DNA-based detection of CRC.

APC protein is another tumor suppressor that assembles on a scaffold protein, axin, with β-catenin and glycogen synthase kinase 3β to coordinate the regulation of β-catenin signaling (24). Inactivation of the APC protein is responsible for both inherited and sporadic forms of CRC. Like K-*ras*, mutation of *APC* appears to be an early-genetic event in the progression from adenoma to carcinoma, which indicates its potential for use as a screening marker. However, unlike K-*ras*, the mutations are distributed throughout the coding region, thereby making it technically difficult and time consuming to detect all of the potential mutations during screening programs for CRC (21, 25, 26).

Microsatellites are stretches of short DNA sequences that contain a motif of 1-5 nucleotides that are tandemly repeated (27). The most common microsatellite in human DNA is a dinucleotide repeat of cytosine and adenine. These tandem repeats occur throughout the human genome. MSI occurs when microsatellites undergo a change in length that covers approximately 15% of the CRC. MSI tumors show a better prognosis than stage-matched microsatellite stable tumors (28). In sporadic CRCs, the development of MSI most commonly occurs due to epigenetic silencing of the DNA mismatch repair gene, *MLH1* (29). There are several MSI markers, among which *BAT26* is probably the most widely used.

Shedding of colonocytes is a normal consequence of exfoliation (30). Untransformed colonocytes are shed continuously from the colonic mucosa. These cells usually undergo apoptosis, which is characterized by DNA fragmentation and laddering. Conversely, malignant colonocytes shed from CRC tumors have a decreased rate of apoptosis relative to normal colonocytes, which facilitates detection of intact genomic DNA (L-DNA) as a potential stool-based marker. Boynton *et al.* (31) amplified six genomic fragments of different length from each of four different genetic loci (*APC, p53, BRCA1, and BRCA2*) using fecal specimens collected from 25 CRC patients and 77 controls. In their study, when a positive L-DNA was defined as >18 bands detected from a possible 24 bands (4 loci × 6 fragments), the specificity for CRC detection was 97% and the sensitivity was 57%.

A large population-based study conducted by Imperiale *et al.* revealed that a fecal DNA panel consisting of 21 mutations (3 in the K-*ras* gene, 10 in the *APC* gene, and 8 in the *p53* gene; the MSI marker BAT-26; and L-DNA) detects a greater proportion of important colorectal neoplasia than FOBT without compromising specificity (32). In addition, the sensitivity of the fecal DNA panel was 52% for invasive caners and 41% for invasive cancers plus adenomas with high-grade dysplasia, whereas that of the FOBT was 13% for the former and 14% for the latter. In subjects with negative findings on colonoscopy, the DNA panel had a specificity of 94%, whereas FOBT had a specificity of 95%. Taken together, the results of this study clearly demonstrate that the DNA panel has a higher sensitivity than FOBT without reduced specificity.

Serum or blood markers

CEA

Carcinoembryonic antigen (CEA) is a high molecular weight glycoprotein that belongs to the immunoglobulin superfamily. The carboxy-terminal of CEA contains a hydrophobic region that is modified to provide a glycosyl phosphatidylinositol link to the cell membrane. Although its presence can be determined in biopsy samples, it is usually identified in serum. This protein has been used for many years as a biomarker of CRCs as well as for other cancers (33). Specifically, high CEA levels are associated with cancer progression, and levels of the marker are expected to fall following cancer surgery (34). However, in the absence of cancer, high CEA levels may also occur in response to other conditions such as hepatitis, inflammatory bowel disease, pancreatitis, and obstructive pulmonary disease. Furthermore, CEA may not be detected when cancer is in advanced stages. As a result, CEA does not provide sufficient sensitivity and reliability for the early detection of cancer. Indeed, using a cut-off point of 2.5 ng/ml results in the sensitivity of CEA for early CRC (i.e., Dukes' A and B disease) being only 30-40% with a specificity of 87% (9). Therefore, the NIH does not recommend that CEA be used to screen for early CRC (33). Instead, the potential value of the CEA test lies in its use to measure the course of the progression of cancer as a prognostic marker once it has been diagnosed, with higher CEA levels being indicative of greater disease severity and a poorer prognosis.

CA 19-9

Carbohydrate antigen 19-9 (CA 19-9), which is the second most investigated gastrointestinal tumor marker, is known to be a sialylated Lewis-a antigen (35). CA 19-9 was originally defined by a monoclonal antibody produced by a hybridoma prepared from the spleen cells of a mouse that had been immunized with the human colorectal carcinoma cell line, SW 1116. Although CA 19-9 is the best marker available for pancreatic adenocarcinoma, it is less sensitive than CEA for CRC and provides less information than CEA when used for monitoring patients that have already been diagnosed with CRC (36). Other carbohydrate antigens such as CA 50, CA 195, CA 242, CA M26, CA M25, CA M43 and CA 72-4 have also been evaluated extensively (37); however, due to their observed sensitivity, stage dependency and specificity, these antigens are not useful markers for the detection of CRC.

Tissue inhibitor of metalloproteinase type 1

Tissue inhibitor of metalloproteinase type 1 (TIMP-1) is a mul-

Exact Sciences Ex. 2003, Page 588 of 1394

tifunctional glycoprotein that inhibits most matrix metalloproteinases (MMPs). The total levels of TIMP-1 have been shown to be significantly higher in patients with both colonic and rectal cancers, while healthy blood donors have a low and very narrow range of plasma TIMP-1 levels (38, 39). More importantly, TIMP-1 is capable of detecting early stage colon cancer with a sensitivity and specificity comparable to those obtained when detecting late stage cancers. Conversely, patients with colon adenomas, inflammatory bowl diseases or primary breast cancer do not show increased plasma levels of total TIMP-1 (40). Preoperative TIMP-1 levels have been proposed as a stage-independent prognostic marker for CRC in multivariate statistical analyses in two separate high-powered studies (41, 42). However, the results of these studies revealed that elevated levels of TIMP-1 were not restricted to advanced stages of CRC alone; therefore, larger studies are required to validate the use of TIMP-1 for early diagnosis and evaluation of the prognosis of CRC.

Five- serum-marker panel (spondin-2, DcR3, Trail-R2, Reg IV, MIC 1)

diaDexus Inc. recently evaluated four serum biomarkers, spondin-2, tumor necrosis factor receptor superfamily member 6B (DcR3), TRAIL receptor 2 (TRAIL-R2) and Reg IV in 600 serum samples in collaboration with the Mayo Clinic (43). All four markers, as well as a fifth marker, macrophage inhibitory cytokine 1 (MIC1), were found to be elevated in patients with CRC when compared to normal controls and patients with benign disease. In addition, this five-serum marker panel offers better sensitivity and specificity than CEA. Accordingly, the company is currently developing biomarker panels that include the 5 markers as diagnostic modalities to improve the detection rate of early stage CRC.

Nicotinamide N-methyltransferase and proteasome activator complex subunit 3

Roche Diagnostics GmbH utilized two-dimensional gel electrophoresis and mass spectrometry to analyze 16 matched CRC and adjacent normal tissue samples. Proteins found to be elevated in cancer tissue were then further validated with serum samples. Elevated levels of nicotinamide N-methyltransferase (NNMT) and proteasome activator complex subunit 3 (PSME3), which are not predicted to be secreted, were found in serum from patients with CRC (44, 45). Validation studies using 109 CRC samples, 317 healthy control samples, and 87 samples from patients with benign bowel diseases revealed that the diagnostic accuracy of PSME3 was similar to that of CEA, and that NNMT was better than CEA at detecting CRC. However, the abundance of PSME3 is less stage-dependent than CEA.

Collapsin response mediator protein-2

Although direct analysis of human samples may sometimes be very challenging, discovering candidate biomarkers from cancer cell lines and subsequent validation in human samples is plausible (46, 47). By analyzing the secretomes of 21 cancer cell lines derived from 12 cancer types, Wu *et al.* identified collapsin response mediator protein-2 (CRMP-2) and evaluated it as a potential CRC biomarker in the serums of 201 CRC patients and 210 healthy controls (48). The use of CRMP-2 alone showed better sensitivity but poorer specificity than CEA. However, combined detection using CEA and CRMP-2 produced better sensitivity (77%) and specificity (95%) than detection using either of these markers alone (43 and 61% sensitivity, respectively; 87 and 65% specificity, respectively). Therefore, CRMP-2 may be a valuable serum marker when used in combination with CEA.

Six SELDI peaks corresponding to apolipoprotein C1, complement C3a-desArg, α1-antitrypsin and transferrin

Surface-enhanced laser desorption/ionization (SELDI) mass spectrometry is a technology that can produce proteomic fingerprints from biological samples using a relatively high-throughput platform (49). In this method, samples are applied to proteinchip arrays coated with chromatographic surfaces and then analyzed by mass spectrometry. Martin *et al.* used SELDI to investigate the serum proteome of 62 CRC patients and 31 noncancer subjects (50). They identified four proteins with diagnostic potential: apolipoprotein C1, complement C3a-desArg (the stable form of C3a anaphylatoxin), α1-antitrypsin and transferrin. Artificial neural networks trained using only the intensities of six SELDI peaks corresponding to the identified proteins were able to classify the patients enrolled in the study with 95% sensitivity and 91% specificity, while the use of CEA to evaluate the same sample set showed 54% sensitivity and 93% specificity.

Habermann *et al.* demonstrated that C3a-desArg is present at significantly higher levels in serum from patients with colorectal adenomas and carcinomas than in serum from healthy individuals (51). Specifically, in a blinded validation study (n = 59), the use of C3a-desArg alone predicted the presence of colorectal malignancy with a sensitivity of 97% and a specificity of 96%. C3a-desArg and other proteins corresponding to SELDI peaks are common serum proteins, and changes in their concentrations most likely reflect epiphenomena rather than secretion by cancer cells.

α-Defensins

Two independent approaches using SELDI-TOF to analyze the protein profiles of colon cancer serum and protein profiles of CRC tumors demonstrated that human neutrophil peptides (HNP)-1, HNP-2 and HNP-3, also known as α-defensin-1, α-defensin-2, and α-defensin-3, are up-regulated in patients with CRC (52, 53). Indeed, the HNP1-3 level in the serum of 48 CRC patients and 42 normal controls was capable of identifying CRC with a sensitivity of 69% and a specificity of 100%. However, a larger study is required to refine and validate the diagnostic accuracy of these findings.

Exact Sciences Ex. 2003, Page 589 of 1394

Macrophage migration inhibitory factor

Based on an observation that the gene expression level of macrophage migration inhibitory factor (MIF) is elevated in CRC tissues, our group has evaluated the use of the protein as a potential biomarker for CRC. In an analysis of serum samples of 129 patients with colon cancer and 53 healthy control subjects, the serum MIF level was found to be significantly increased in patients with CRC (54). Although the specificity of MIF is not as high as that of CEA (90.6% vs. 100.0%), MIF is more sensitive during early cancer detection (47.3% vs. 29.5%), which suggests that MIF may be used as a diagnostic marker in CRC.

Macrophage-colony stimulating factor

The serum levels of both macrophage-colony stimulating factor (M-CSF) and granulocyte-colony stimulating factor are significantly higher in CRC patients than in healthy subjects (55, 56). In addition, serum levels of M-CSF are more associated with lymph node metastasis than CEA and CA 19-9, which suggests that serum M-CSF elevation in CRC patients might help predict the risk of lymph node metastasis of this tumor. Finally, M-CSF appears to offer additional information to that presented by classic prognostic factors.

Prolactin

Prolactin, which is a hormone with multiple biological actions that is synthesized by the anterior pituitary gland, is elevated in patients with CRC. A study that evaluated 47 CRC patients and 51 healthy controls revealed that prolactin can predict CRC with a sensitivity and specificity of 77% and 98%, respectively (57).

M2-pyruvate kinase

M2-pyruvate kinase is an isoform of glycolytic enzyme pyruvate kinase. Although the protein is a cytosolic enzyme, it is liberated into circulation via a mechanism that is not yet known. However, it has been suggested that M2-pyruvate kinase is released into circulation from dying cancer cells. Therefore, M2-pyruvate may be a useful marker for the detection of CRC. In addition, two independent studies revealed that the use of M2-pyruvate kinase for the detection of CRC has a sensitivity of 48-58% and a specificity of 90-95%. Furthermore, when combined with CEA, the sensitivity of M2-pyruvate increases without decreasing the specificity (58, 59).

Methylated septin-9 DNA

The assessment of epigenetic events is one of the most promising means of identifying biomarker candidates for the early detection of cancer. DNA methylation in which cytosines within the palindromic dinucleotide 5'-CpG-3' sequence are methylated shapes the chromatin structure of DNA according to its functional state (60). The cancer genome is frequently characterized by hypermethylation of specific genes. Therefore, Epigenomics AG has developed a blood-test for CRC that is based on methylation of *SEPT9*, *NGFR* and *TMEEF2* (61). To evaluate this test, free-floating DNA was extracted from plasma samples of 133 CRC patients and 179 healthy controls in the same age range, and the methylation levels were then measured using restriction enzyme-based qPCR. The biomarker with the highest performance was found to be *SEPT9*, which was capable of detecting CRC with a specificity and sensitivity of 95% and 52%, respectively, when a cutoff of 0.011 μg/L of methylated *SEPT9* DNA was used.

Five gene markers in whole blood

Gene expression patterns in the peripheral blood reflect changes that occur within the cells and tissues of the body (62). Han *et al.* extracted total RNA from the white blood cells of peripheral blood and identified differentially regulated genes using a microarray (63). Specifically, they used a panel comprised of five genes including B-cell scaffold protein with ankyrin repeats 1 (BANK1), B-cell novel protein 1 (*BCNP1*), cytidine deaminase (*CDS*), FERM domain containing 3 (*MGC20553*), and membrane-spanning 4-domains, subfamily A, member 1 (*MS4A1*) to detect CRC and found that this test had a sensitivity of 88-94% and a specificity of 64-77%.

Other markers

Three proteins, colon cancer-specific antigen (CCSA)-2, CCSA-3 and CCSA-4, have shown promise as markers for the detection of CRC. Getzenberg *et al.* identified several nuclear matrix proteins from CRC cell lines and tested some of them to determine if they would be useful as cancer biomarkers (64). Using a cutoff value of 2 μg/ml for CCSA-3, both CRC and advanced adenoma were detected with 89% sensitivity and 82% specificity. In addition, the sensitivity and specificity was 85% and 91%, respectively, when CCSA-4 was used with a cutoff value of 0.3 μg/ml. Finally, the use of CCSA-2 at a cutoff of 10.8 μg/ml had an overall specificity of 78% and sensitivity of 97% when used to separate individuals with advanced adenomas and CRC from normal, hyperplastic, and nonadvanced adenoma populations (65). Although the initial studies have had promising outcomes, the molecular identities of the three proteins have not yet been publically disclosed.

Remodeling of the extracellular matrix is important in the development of cancers, and several extracellular matrix proteins that can be liberated into circulation have been evaluated as potential biomarkers. The results of these evaluations have revealed that the serum levels of MMP9 and MMP7 depend on the presence of colorectal malignancy (66, 67). In addition, it has been suggested that serum laminin and MMP7 can be used as independent prognostic markers of CRC (67, 68).

Conclusion and perspectives

FOBT is currently the only screening modality for CRC. DNA-based fecal markers are promising but are not widely

Exact Sciences Ex. 2003, Page 590 of 1394

used in clinical settings. In addition, a lack of sensitivity and specificity preclude the use of all existing serum markers for the early detection of CRC. CEA is used to monitor therapy in advanced CRC, and the preoperative level of CEA is used to provide prognostic information. However, there is insufficient evidence for routine use of other classic serum markers such as carbohydrate antigens and TIMP-1. Therefore, large-scale validation studies are required to evaluate the potential for the use of biomarkers that have recently been discovered through omics technology.

Genomics, proteomics, and a combination of both of these methods play major roles in the discovery of biomarkers. As with many other methods, each approach has its advantages and disadvantages. However, in light of the fact that many important functions of proteins require post-translational modification or their interaction with other protein(s), proteomics will continue to play a dominant role in the biomarker field. Clinical proteomics as a principal tool for the detection of differentially expressed proteins in samples from CRC patients versus healthy donors is required to significantly increase the repertoire of candidate biomarkers. In addition, steps should be taken to design adequate clinical trials and build a business model that can be used for the commercialization of newly discovered biomarkers. These efforts will hasten the translation of proteomic discoveries into clinical practice in the not-so-distant future.

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REFERENCES

1. Bingham, S. and Riboli, E. (2004) Diet and cancer–the European Prospective Investigation into Cancer and Nutrition. *Nat. Rev. Cancer* **4**, 206-215.
2. Sung, J. J., Lau, J. Y., Goh, K. L., Leung, W. K. and Asia Pacific Working Group on Colorectal Cancer (2005) Increasing incidence of colorectal cancer in Asia: implications for screening. *Lancet Oncol.* **6**, 871-876.
3. Hardcastle, J. D., Chamberlain, J. O., Robinson, M. H., Moss, S. M., Amar, S. S., Balfour, T. W., James, P. D. and Mangham, C. M. (1996) Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* **348**, 1472-1477.
4. Kronborg, O., Fenger, C., Olsen, J., Jorgensen, O. D. and Sondergaard, O. (1996) Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* **348**, 1467-1471.
5. Rubenstein, K. (2007) *Disease-related biomarkers: their potential in patient screening, prognosis, and stratification*, Insight Pharma Reports, Needham, MA, USA
6. Geenen, J. E., Schmitt, M. G., Jr., Wu, W. C. and Hogan, W. J. (1975) Major complications of coloscopy: bleeding and perforation. *Am. J. Dig. Dis.* **20**, 231-235.
7. Winawer, S., Fletcher, R., Rex, D., Bond, J., Burt, R., Ferrucci, J., Ganiats, T., Levin, T., Woolf, S., Johnson, D., Kirk, L., Litin, S. and Simmang, C. (2003) Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology* **124**, 544-560.
8. Booth, R. A. (2007) Minimally invasive biomarkers for detection and staging of colorectal cancer. *Cancer Lett.* **249**, 87-96.
9. Duffy, M. J., van Dalen, A., Haglund, C., Hansson, L., Klapdor, R., Lamerz, R., Nilsson, O., Sturgeon, C. and Topolcan, O. (2003) Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines. *Eur. J. Cancer* **39**, 718-727.
10. Habermann, J. K., Bader, F. G., Franke, C., Zimmermann, K., Gemoll, T., Fritzsche, B., Ried, T., Auer, G., Bruch, H. P. and Roblick, U. J. (2008) From the genome to the proteome-biomarkers in colorectal cancer. *Langenbecks Arch. Surg.* **393**, 93-104.
11. Kim, S. Y. and Hahn, W. C. (2007) Cancer genomics: integrating form and function. *Carcinogenesis* **28**, 1387-1392.
12. Faca, V., Krasnoselsky, A. and Hanash, S. (2007) Innovative proteomic approaches for cancer biomarker discovery. *BioTechniques* **43**, 279, 281-273, 285.
13. Duffy, M. J., van Dalen, A., Haglund, C., Hansson, L., Holinski-Feder, E., Klapdor, R., Lamerz, R., Peltomaki, P., Sturgeon, C. and Topolcan, O. (2007) Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *Eur. J. Cancer* **43**, 1348-1360.
14. Huang, C. S., Lal, S. K. and Farraye, F. A. (2005) Colorectal cancer screening in average risk individuals. *Cancer Causes Control* **16**, 171-188.
15. Mandel, J. S., Bond, J. H., Church, T. R., Snover, D. C., Bradley, G. M., Schuman, L. M. and Ederer, F. (1993) Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N. Engl. J. Med.* **328**, 1365-1371.
16. Loktionov, A., O'Neill, I. K., Silvester, K. R., Cummings, J. H., Middleton, S. J. and Miller, R. (1998) Quantitation of DNA from exfoliated colonocytes isolated from human stool surface as a novel noninvasive screening test for colorectal cancer. *Clin. Cancer Res.* **4**, 337-342.
17. Shaw, R. J. and Cantley, L. C. (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* **441**, 424-430.
18. Fearon, E. R. and Vogelstein, B. (1990) A genetic model for colorectal tumorigenesis. *Cell* **61**, 759-767.
19. Losi, L., Roncucci, L., di Gregorio, C., de Leon, M. P. and Benhattar, J. (1996) K-ras and p53 mutations in human colorectal aberrant crypt foci. *J. Pathol.* **178**, 259-263.
20. Shivapurkar, N., Huang, L., Ruggeri, B., Swalsky, P. A., Bakker, A., Finkelstein, S., Frost, A. and Silverberg, S. (1997) K-ras and p53 mutations in aberrant crypt foci and colonic tumors from colon cancer patients. *Cancer Lett.* **115**, 39-46.
21. Smith, A. J., Stern, H. S., Penner, M., Hay, K., Mitri, A., Bapat, B. V. and Gallinger, S. (1994) Somatic APC and

Exact Sciences Ex. 2003, Page 591 of 1394

K-ras codon 12 mutations in aberrant crypt foci from human colons. *Cancer Res.* **54**, 5527-5530.
22. Mills, A. A. (2005) p53: link to the past, bridge to the future. *Genes Dev.* **19**, 2091-2099.
23. Iacopetta, B. (2003) TP53 mutation in colorectal cancer. *Hum. Mutat.* **21**, 271-276.
24. Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B. and Polakis, P. (1998) Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. *Curr. Biol.* **8**, 573-581.
25. Srivastava, S., Verma, M. and Henson, D. E. (2001) Biomarkers for early detection of colon cancer. *Clin. Cancer Res.* **7**, 1118-1126.
26. Ahlquist, D. A., Skoletsky, J. E., Boynton, K. A., Harrington, J. J., Mahoney, D. W., Pierceall, W. E., Thibodeau, S. N. and Shuber, A. P. (2000) Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* **119**, 1219-1227.
27. Dietmaier, W., Wallinger, S., Bocker, T., Kullmann, F., Fishel, R. and Ruschoff, J. (1997) Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res.* **57**, 4749-4756.
28. Ribic, C. M., Sargent, D. J., Moore, M. J., Thibodeau, S. N., French, A. J., Goldberg, R. M., Hamilton, S. R., Laurent-Puig, P., Gryfe, R., Shepherd, L. E., Tu, D., Redston, M. and Gallinger, S. (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N. Engl. J. Med.* **349**, 247-257.
29. Esteller, M., Levine, R., Baylin, S. B., Ellenson, L. H. and Herman, J. G. (1998) MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* **17**, 2413-2417.
30. Albaugh, G. P., Iyengar, V., Lohani, A., Malayeri, M., Bala, S. and Nair, P. P. (1992) Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. *Int. J. Cancer* **52**, 347-350.
31. Boynton, K. A., Summerhayes, I. C., Ahlquist, D. A. and Shuber, A. P. (2003) DNA integrity as a potential marker for stool-based detection of colorectal cancer. *Clin. Chem.* **49**, 1058-1065.
32. Imperiale, T. F., Ransohoff, D. F., Itzkowitz, S. H., Turnbull, B. A. and Ross, M. E. (2004) Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N. Engl. J. Med.* **351**, 2704-2714.
33. Bates, J. (2008) *Cancer biomarkers: adoption is driving growth*, Insight Pharma Reports, Needham, MA, USA
34. Duffy, M. J. (2001) Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? *Clin. Chem.* **47**, 624-630.
35. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H. and Ginsburg, V. (1982) A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J. Biol. Chem.* **257**, 14365-14369.
36. Duffy, M. J. (1998) CA 19-9 as a marker for gastrointestinal cancers: a review. *Ann. Clin. Biochem.* **35 (Pt 3)**, 364-370.
37. Hundt, S., Haug, U. and Brenner, H. (2007) Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiol. Biomarkers Prev.* **16**, 1935-1953.
38. Holten-Andersen, M. N., Murphy, G., Nielsen, H. J., Pedersen, A. N., Christensen, I. J., Hoyer-Hansen, G., Brunner, N. and Stephens, R. W. (1999) Quantitation of TIMP-1 in plasma of healthy blood donors and patients with advanced cancer. *Br. J. Cancer* **80**, 495-503.
39. Sorensen, N. M., Schrohl, A. S., Jensen, V., Christensen, I. J., Nielsen, H. J. and Brunner, N. (2008) Comparative studies of tissue inhibitor of metalloproteinases-1 in plasma, serum and tumour tissue extracts from patients with primary colorectal cancer. *Scand. J. Gastroenterol.* **43**, 186-191.
40. Holten-Andersen, M. N., Fenger, C., Nielsen, H. J., Rasmussen, A. S., Christensen, I. J., Brunner, N. and Kronborg, O. (2004) Plasma TIMP-1 in patients with colorectal adenomas: a prospective study. *Eur. J. Cancer* **40**, 2159-2164.
41. Holten-Andersen, M., Christensen, I. J., Nilbert, M., Bendahl, P. O., Nielsen, H. J., Brunner, N. and Fernebro, E. (2004) Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival. a validation study. *Eur. J. Cancer* **40**, 64-72.
42. Holten-Andersen, M. N., Stephens, R. W., Nielsen, H. J., Murphy, G., Christensen, I. J., Stetler-Stevenson, W. and Brunner, N. (2000) High preoperative plasma tissue inhibitor of metalloproteinase-1 levels are associated with short survival of patients with colorectal cancer. *Clin. Cancer Res.* **6**, 4292-4299.
43. diaDexus Inc. (2007) Clinical application of diaDexus diagnostics in colorectal cancer http://www.diadexus.com/products/research/oncoogy_diagnostics.php
44. Roessler, M., Rollinger, W., Palme, S., Hagmann, M. L., Berndt, P., Engel, A. M., Schneidinger, B., Pfeffer, M., Andres, H., Karl, J., Bodenmuller, H., Ruschoff, J., Henkel, T., Rohr, G., Rossol, S., Rosch, W., Langen, H., Zolg, W. and Tacke, M. (2005) Identification of nicotinamide *N*-methyltransferase as a novel serum tumor marker for colorectal cancer. *Clin. Cancer Res.* **11**, 6550-6557.
45. Roessler, M., Rollinger, W., Mantovani-Endl, L., Hagmann, M. L., Palme, S., Berndt, P., Engel, A. M., Pfeffer, M., Karl, J., Bodenmuller, H., Ruschoff, J., Henkel, T., Rohr, G., Rossol, S., Rosch, W., Langen, H., Zolg, W. and Tacke, M. (2006) Identification of PSME3 as a novel serum tumor marker for colorectal cancer by combining two-dimensional polyacrylamide gel electrophoresis with a strictly mass spectrometry-based approach for data analysis. *Mol. Cell. Proteomics* **5**, 2092-2101.
46. Volmer, M. W., Stuhler, K., Zapatka, M., Schoneck, A., Klein-Scory, S., Schmiegel, W., Meyer, H. E. and Schwarte-Waldhoff, I. (2005) Differential proteome analysis of conditioned media to detect Smad4 regulated secreted biomarkers in colon cancer. *Proteomics* **5**, 2587-2601.
47. Chang, J. W., Kang, U.-B., Kim, D. H., Yi, J. K., Lee, J. W., Noh, D.-Y., Lee, C. and Yu, M.-H. (2008) Identification of

Exact Sciences Ex. 2003, Page 592 of 1394

circulating endorepellin LG3 fragment: Potential use as a serological biomarker for breast cancer. *Proteomics Clin. Appl.* **2**, 23-32.
48. Wu, C. C., Chen, H. C., Chen, S. J., Liu, H. P., Hsieh, Y. Y., Yu, C. J., Tang, R., Hsieh, L. L., Yu, J. S. and Chang, Y. S. (2008) Identification of collapsin response mediator protein-2 as a potential marker of colorectal carcinoma by comparative analysis of cancer cell secretomes. *Proteomics* **8**, 316-332.
49. Engwegen, J. Y., Gast, M. C., Schellens, J. H. and Beijnen, J. H. (2006) Clinical proteomics: searching for better tumour markers with SELDI-TOF mass spectrometry. *Trends Pharmacol. Sci.* **27**, 251-259.
50. Ward, D. G., Suggett, N., Cheng, Y., Wei, W., Johnson, H., Billingham, L. J., Ismail, T., Wakelam, M. J., Johnson, P. J. and Martin, A. (2006) Identification of serum biomarkers for colon cancer by proteomic analysis. *Br. J. Cancer* **94**, 1898-1905.
51. Habermann, J. K., Roblick, U. J., Luke, B. T., Prieto, D. A., Finlay, W. J., Podust, V. N., Roman, J. M., Oevermann, E., Schiedeck, T., Homann, N., Duchrow, M., Conrads, T. P., Veenstra, T. D., Burt, S. K., Bruch, H. P., Auer, G. and Ried, T. (2006) Increased serum levels of complement C3a anaphylatoxin indicate the presence of colorectal tumors. *Gastroenterology* **131**, 1020-1029; quiz 1284.
52. Albrethsen, J., Bogebo, R., Gammeltoft, S., Olsen, J., Winther, B. and Raskov, H. (2005) Upregulated expression of human neutrophil peptides 1, 2 and 3 (HNP 1-3) in colon cancer serum and tumours: a biomarker study. *BMC Cancer* **5**, 8.
53. Melle, C., Ernst, G., Schimmel, B., Bleul, A., Thieme, H., Kaufmann, R., Mothes, H., Settmacher, U., Claussen, U., Halbhuber, K. J. and Von Eggeling, F. (2005) Discovery and identification of alpha-defensins as low abundant, tumor-derived serum markers in colorectal cancer. *Gastroenterology* **129**, 66-73.
54. Lee, H., Rhee, H., Kang, H. J., Kim, H. S., Min, B. S., Kim, N. K. and Kim, H. (2008) Macrophage migration inhibitory factor may be used as an early diagnostic marker in colorectal carcinomas. *Am. J. Clin. Pathol.* **129**, 772-779.
55. Mroczko, B., Groblewska, M., Wereszczynska-Siemiatkowska, U., Okulczyk, B., Kedra, B., Laszewicz, W., Dabrowski, A. and Szmitkowski, M. (2007) Serum macrophage-colony stimulating factor levels in colorectal cancer patients correlate with lymph node metastasis and poor prognosis. *Clin. Chim. Acta* **380**, 208-212.
56. Mroczko, B., Groblewska, M., Wereszczynska-Siemiatkowska, U., Kedra, B., Konopko, M. and Szmitkowski, M. (2006) The diagnostic value of G-CSF measurement in the sera of colorectal cancer and adenoma patients. *Clin. Chim. Acta* **371**, 143-147.
57. Soroush, A. R., Zadeh, H. M., Moemeni, M., Shakiba, B. and Elmi, S. (2004) Plasma prolactin in patients with colorectal cancer. *BMC Cancer* **4**, 97.
58. Schneider, J., Bitterlich, N. and Schulze, G. (2005) Improved sensitivity in the diagnosis of gastro-intestinal tumors by fuzzy logic-based tumor marker profiles including the tumor M2-PK. *Anticancer Res.* **25**, 1507-1515.
59. Zhang, B., Chen, J. Y., Chen, D. D., Wang, G. B. and Shen, P. (2004) Tumor type M2 pyruvate kinase expression in gastric cancer, colorectal cancer and controls. *World J. Gastroenterol.* **10**, 1643-1646.
60. Zhu, J. and Yao, X. (2007) Use of DNA methylation for cancer detection and molecular classification. *J. Biochem. Mol. Biol.* **40**, 135-141.
61. Lofton-Day, C., Model, F., Devos, T., Tetzner, R., Distler, J., Schuster, M., Song, X., Lesche, R., Liebenberg, V., Ebert, M., Molnar, B., Grutzmann, R., Pilarsky, C. and Sledziewski, A. (2008) DNA methylation biomarkers for blood-based colorectal cancer screening. *Clin. Chem.* **54**, 414-423.
62. Liew, C. C., Ma, J., Tang, H. C., Zheng, R. and Dempsey, A. A. (2006) The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J. Lab. Clin. Med.* **147**, 126-132.
63. Han, M., Liew, C. T., Zhang, H. W., Chao, S., Zheng, R., Yip, K. T., Song, Z. Y., Li, H. M., Geng, X. P., Zhu, L. X., Lin, J. J., Marshall, K. W. and Liew, C. C. (2008) Novel blood-based, five-gene biomarker set for the detection of colorectal cancer. *Clin. Cancer Res.* **14**, 455-460.
64. Brunagel, G., Vietmeier, B. N., Bauer, A. J., Schoen, R. E. and Getzenberg, R. H. (2002) Identification of nuclear matrix protein alterations associated with human colon cancer. *Cancer Res.* **62**, 2437-2442.
65. Leman, E. S., Schoen, R. E., Magheli, A., Sokoll, L. J., Chan, D. W. and Getzenberg, R. H. (2008) Evaluation of colon cancer-specific antigen 2 as a potential serum marker for colorectal cancer. *Clin. Cancer Res.* **14**, 1349-1354.
66. Hurst, N. G., Stocken, D. D., Wilson, S., Keh, C., Wakelam, M. J. and Ismail, T. (2007) Elevated serum matrix metalloproteinase 9 (MMP-9) concentration predicts the presence of colorectal neoplasia in symptomatic patients. *Br. J. Cancer* **97**, 971-977.
67. Maurel, J., Nadal, C., Garcia-Albeniz, X., Gallego, R., Carcereny, E., Almendro, V., Marmol, M., Gallardo, E., Maria Auge, J., Longaron, R., Martinez-Fernandez, A., Molina, R., Castells, A. and Gascon, P. (2007) Serum matrix metalloproteinase 7 levels identifies poor prognosis advanced colorectal cancer patients. *Int. J. Cancer* **121**, 1066-1071.
68. Saito, N. and Kameoka, S. (2005) Serum laminin is an independent prognostic factor in colorectal cancer. *Int. J. Colorectal Dis.* **20**, 238-244.

Exact Sciences Ex. 2003, Page 593 of 1394

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	53	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:50
S2	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same "ng/ml" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:51
S3	9	(hemoglobin haemoglobin) with concentration same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:18
S4	260	DNA same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:35
S5	2	"20080227208"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:34
S6	1	"20080227208" and "10 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:35
S7	11	(hemoglobin haemoglobin) with "5 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:38
S8	0	NDGR4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:50
S9	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR;	OR	OFF	2020/01/14 13:50

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S10	6	NDRG4 same epigenetic and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:58
S11	24	"2006113770"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:02
S12	4	"2006113770" and NDRG4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:11
S13	6	NDRG4 same (epigenetic methylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S14	6	NDRG4 same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S15	0	"N-myc downstream regulated gene 4" same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S16	0	"N-myc downstream regulated gene 4" and (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S17	0	"N-myc downstream regulated gene 4" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:25
S18	1	"20060019256" and uptg	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:55

S19	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 15:35
S20	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:07
S21	3	fecal same (antibody antibodies immunoassay) same (DNA RNA polynucleotide "nucleic acid" gene) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:26
S22	2	(cancer carcinoma) same (PHACTR3 NDRG4 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFRP2 CDO1) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:40
S23	1	"200483399"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:42
S24	0	"200483399A2"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:43
S25	0	"200483399A2"	EPO	OR	OFF	2020/01/15 11:11
S26	0	"200483399"	EPO	OR	OFF	2020/01/15 11:11
S27	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 18:14
S28	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 19:10
S29	15	hemoglobin with (stool fecal) with concentration and @pd<"20090203"	USPAT	OR	OFF	2021/06/15 10:13

S30	32	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and (antibody antibodies immunoassay immunological) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:33
S31	16	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:33
S32	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and "ng/ml" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:37
S33	1,048	(freeze freezing frozen) same (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:05
S34	399	(freeze freezing frozen) with (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:05
S35	236	(freeze freezing frozen) with (stool fecal faecal) with (sample fraction portion historical) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:07
S36	14	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion historical) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:07
S37	4	(immunoassay elisa) with (stool fecal faecal) with (fresh "non-frozen") and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:31
S38	1	"7832219".FMID.	US-PGPUB; USPAT; FPRS; JPO	OR	OFF	2021/06/16 12:32
S39	4	"7832219"	USPAT	OR	ON	2021/06/16 12:33
S40	2	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	ON	2021/06/16 13:30

		historical) same buffer and @pd<"20090203"	DERWENT; IBM_TDB			
S41	5	(freeze freezing frozen) same (stool fecal faecal) with (fraction portion historical) same buffer and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 13:33
S42	230	"5741650"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 13:38
S43	114	"5741650"	USPAT	OR	ON	2021/06/16 13:38
S44	63	"5741650" and frozen	USPAT	OR	ON	2021/06/16 13:40
S45	65	"5741650" and (freeze freezing frozen)	USPAT	OR	ON	2021/06/16 13:41
S46	3	(feces fecal faecal faeces) with buffer with (freeze freezing frozen) and @pd<"20090203"	USPAT	OR	ON	2021/06/16 13:51

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15634607	CTNF	06/28/2021	EXCTD-35239.304
	892	06/28/2021	EXCTD-35239.304

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION MAILED 06/28/2021

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF EFS WEB TRANSMISSION UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being transmitted to the United States Patent and Trademark Office transmitted via the Office electronic filing system in accordance with 37 C.F.R. §1.6(a)(4).

Dated: November 29, 2021 By: /Mary Ann D. Brow/
Mary Ann D. Brow, Reg. No. 42,363

This paper is responsive to the Office Action mailed 06/28/2021, with response due by 09/28/2021. A two-month extension of time, to Monday, 11/29/2021, the first business day following Sunday, 11/28/2021, is requested. Applicant respectfully requests reconsideration in view of the amendments and remarks hereinbelow.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: **EXCTD-35239.304**. This paragraph is a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Amendments to the Claims are reflected in the Listing of Claims beginning on Page 2;

Remarks begin on Page 5.

AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior listings and versions of claims in the application:

1-81. (canceled)

82. (currently amended) A method of processing a freshly-collected fecal sample without freezing, the method comprising:

a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;

b) removing a portion of said fecal sample to a separate container to produce a removed portion and a remaining portion of said fecal sample;

c) combining the removed portion in the separate container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample; and

d) stabilizing the remaining portion of the fecal sample by adding a buffer that maintains DNA integrity in a fecal sample to the remaining portion in the sealable collection vessel.

83. (canceled)

84. (currently amended) The method of claim 82, further comprising delivering the container containing the removed portion and buffer and the sealable vessel containing the remaining portion of the fecal sample and buffer to a medical diagnostics laboratory.

85-86. (canceled)

87. (currently amended) A method of processing a fecal sample, the method comprising:

A) obtaining a pair of portions of a fecal sample from a human subject, the pair of

portions comprising a container containing a removed portion and a buffer and a sealable vessel containing a stabilized remaining portion of a fecal sample and a buffer obtained by the method of claim 82, ~~and~~

B) testing the removed portion of said fecal sample for ~~the presence of blood~~ a concentration of hemoglobin present in said removed portion,

C) extracting DNA from the remaining portion of said fecal sample; and

D) analyzing said DNA for the presence of DNA from the human subject having an epigenetic modification.

88. (canceled)

89. (previously presented) The method of claim 87, wherein testing for the concentration of hemoglobin in said removed portion comprises immunochemical detection of hemoglobin.

90. (currently amended) The method of claim ~~88~~87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 5 ng/ml.

91. (currently amended) The method of claim ~~88~~87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 10 ng/ml.

92. (currently amended) The method of claim ~~88~~87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 20 ng/ml.

93. (currently amended) The method of claim ~~88~~87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 50 ng/ml.

94. (currently amended) The method of claim ~~88~~87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 200 ng/ml.

95. (canceled)

96. (currently amended) The method of claim ~~95~~87, wherein said epigenetic modification comprises aberrant methylation.

97. (currently amended) The method of claim ~~95~~87, wherein said aberrant methylation comprises hypermethylation.

98. (currently amended) The method of claim ~~95~~87, wherein said DNA from ~~a~~ the human subject comprises a gene and/or a promoter region of a gene.

99. (previously presented) The method of claim 98, wherein said gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.

100. (previously presented) The method of claim 96, wherein analyzing said DNA comprises modifying said DNA with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil.

REMARKS

In the Office Action mailed 06/28/2021 ("Office Action"), the Examiner made the following objections and rejections:

I. Claims 82 and 83 are objected to because of the following informalities: In claim 82, there is no conjunctive (and) or disjunctive (or) following step c), and before step d). Claim 83 lacks a conjunctive or disjunctive following step i), but before step ii).

II. Claims 82-83, and 87 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41, hereinafter "Kutzner") in view of US 2008/0124714 Al ("Shuber") and US 2006/0216830 Al ("Kikuiri").

III. Claims 87-94 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner in view of Shuber as applied to claim 82, and further in view of Kikuiri and US 2008/0227298 Al (Yee).

IV. Claims 87, 95-100 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner in view of Shuber as applied to claim 87, and further in view of either Kim (BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008; hereinafter "Kim") or WO 2008/084219 Al ("Van Engeland").

Claim amendments

For business reasons and without acquiescing to the Examiner's arguments, and reserving the right to prosecute the original or similar claims in one or more future applications, claim 82 is herein amended to recite a method of processing a freshly collected fecal sample without freezing, wherein the fecal sample is collected at home by a human subject by defecation directly into a sealable collection vessel. The removed portion is removed to a separate container and combined with a buffer and a buffer that maintains DNA integrity is added to the remaining portion in the sealable collection vessel. Support for the amendment is found in the application as originally filed, e.g., at page 28, second paragraph; the paragraph bridging pages 38-39; the

paragraph bridging pages 43-44.

Claim 83 is canceled without prejudice and claim 84 is amended for consistency with claim 82.

Claim 87 is amended to incorporate the elements of claims 88 and 95, which are cancelled in view of the amendment to claim 87.

Claims 90-94 and 96-98 are amended to depend from claim 87 instead of cancelled claims 88 and 95.

These amendments do not add new matter.

Objections

I. Claims 82 and 83 are objected to because of the following alleged informalities:

- In claim 82, there is no conjunctive (and) or disjunctive (or) following step c), and before step d). Claim 83 lacks a conjunctive or disjunctive following step i), but before step ii).

Claim 82 as herein amended recites "and" following step c) and before step d). Claim 83 is canceled in the present amendment, rendering the objection moot.

The claims are not obvious

II. Claims 82-83, and 87 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner in view of Shuber and Kikuiri. Applicant respectfully disagrees that the cited references render the claims *prima facie* obvious.

It is well-settled law that establishing *prima facie* obviousness requires *at least* a suggestion of all of the features in a claim. *See In re Wada and Murphy, citing CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) and *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). See also MPEP §2143. Further, a valid *prima facie* case requires the Office to present "articulated reasoning with some rational underpinning", *KSR*, 550 U.S. at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)), that one of skill in the art would have been motivated to combine the elements to yield the claimed combination and that one would have done so having a reasonable expectation of successfully carrying out the claimed combination. *In*

re Vaeck, 947 F.2d 488, 493 (Fed. Cir. 1991).

As discussed above, the instant claims as amended are directed to a method of processing a freshly-collected fecal sample from a subject without freezing, wherein the sample is collected at home by the subject by defecation directly into a sealable collection vessel, wherein the sample is divided into portions that are separately stabilized, *e.g.*, for preserving different types of analytes in each portion. In particular, a removed portion in a separate container is combined with a buffer that stabilizes blood proteins and the remaining portion is separately treated in the original collection vessel with a buffer that maintains DNA integrity in the fecal sample. A fecal sample collected in the claimed manner is suitable for detecting blood proteins in one portion and DNA in the other portion of the same fecal sample.

The Examiner points to Kutzner as disclosing detection of colorectal tumors using a combination of tests, one which looks for a component of blood, and one which is based on nucleic acid analysis, e.g., PCR analysis. (Office Action, ¶14).

While not acquiescing to the Examiner's characterization of Kutzner, Applicant points out that Kutzner describes a retrospective study of mutations and blood in frozen patient samples (Kutzner, p34, col 2). Kutzner does not teach or suggest a method of processing a freshly-collected fecal sample without freezing using a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, as recited in instant claim 82. As acknowledged by the Examiner, Kutzner also does not suggest testing a fecal sample for a concentration of hemoglobin, much less testing a removed portion collected as described in the instant claims for a concentration of hemoglobin. The Examiner also acknowledges that Kutzner does not teach assaying DNA for aberrant methylation.

Further, Kutzner clearly states that their "retrospective data *do not allow any conclusion* on the potential of this approach [combined application of molecular diagnosis (MD) and fecal occult blood test (FOBT)] in diagnostic screening" and state that "Prospective comparative studies, which include a very high number of individuals, have to prove the value of the combined application of MD and FOBT." (Kutzner, p40, paragraph bridging columns 1-2,

emphasis added). Thus, Kutzner specifically states that it is not obvious to the authors of the reference, as skilled artisans, that combined testing of MD markers with FOBT will yield any particular result with respect to diagnostic screening. Kutzner provides no more than a guess that the approach *might be* successful, but does not provide a "reasonable expectation of success" required to establish *prima facie* obviousness.

Combination with Shuber and Kikuiri does not cure the deficiencies of Kutzner. While Shuber provides a number of methods of stabilizing nucleic acids, Shuber does not teach a method of processing a freshly-collected fecal sample without freezing using a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample.

While Kikuiri discloses a device for collecting a sample of feces for hemoglobin testing, the device of Kikuiri is not configured to collect a fecal sample from a human subject by defecation directly into the container. See, for example, Fig. 4 of Kikuiri, illustrating specimen sampling member 20, "pinched with fingers" and showing specimen-sampling grooves 23*b* at the distal end. (see Kikuiri, ¶49). This illustration shows that the sample contemplated by Kikuiri is delivered into the container using a sample collecting member, rather than by defecation into the container. Further, Kikuiri make no suggestion of a buffer that "stabilizes blood proteins." Rather, Kikuiri also discloses only a "feces-dissolving liquid".

The combination of Kutzner, Shuber, and Kikuiri does not teach or suggest processing a freshly-collected fecal sample without freezing using a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample.

While not acquiescing that the other requirements for establishing *prima facie* obviousness have been met, Applicant submits that, for the reasons set forth above, Kutzner in

view of Shuber and Kikuiri does not set forth all the elements of claims 82-83, and 87 and for at least this reason does not render these claims *prima facie* obvious. Applicant therefore respectfully requests that the rejection be withdrawn.

III. Claims 87-94 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner in view of Shuber as applied to claim 82, and further in view of Kikuiri and Yee.

The combination of Kutzner, Shuber, and Kikuiri as applied to claim 87 is discussed above. Claim 88 is canceled in the present amendment and remaining claims 89-94 depend from claim 87 and thereby comprise all the same elements of claim 87. As discussed in detail below, Yee does not cure the deficiencies of Kutzner, Shuber, and Kikuiri.

The Examiner admits that Kutzer in view of Shuber does not teach limitations such as delivering fecal samples and separate portions to a lab, or hemoglobin detection with various concentrations (Office Action, ¶21). The Examiner points to Kikuiri as teaching a container in which a fecal specimen can be transported. However, as discussed above, the device of Kikuiri is not configured to collect a fecal sample from a human subject by defecation directly into the container, or for transportation of a buffer-stabilized "remaining portion" to a medical facility for testing.

The Examiner further asserts that "it would have also been obvious to an ordinary artisan to have employed distinct portions from a subject's fecal sample in testing lab, as exemplified by Kikuiri, to detect multiple signals indicative of colorectal cancer or tumor, e.g., an inclusion of detecting occult blood (hemoglobin) at various desired contractions based on known threshold levels." (Office Action, ¶27).

Applicant disagrees with the Examiner's characterization of Kikuiri. While Kikuiri discloses a device for collecting a sample of feces for hemoglobin testing, Kikuiri makes no mention of what happens to any portion of the feces that is not collected into the disclosed device. Kikuiri makes no mention of employing distinct portion**s (plural)** from a subject's fecal sample in a testing lab, nor of "detecting **multiple signals** indicative of colorectal cancer or tumor," as alleged by the Examiner.

The Examiner further asserts that "The ordinary artisan would have been motivated

perform multiple testing on different targets present in a fecal sample in order to enhance various degrees of accurate diagnosis of colorectal cancer, as taught and suggested by the combined cited references" (Office Action, ¶27). However, this statement is not supported by the cited references or by any other evidence. As noted above, Kutzner clearly states that their "retrospective data *do not allow any conclusion* on the potential of this approach in diagnostic screening" and that prospective comparative studies that include very high number of individuals would be required to prove the value of the combined application of molecular testing and FOBT. (Kutzner, p40, paragraph bridging columns 1-2). Thus, it is not obvious even to the authors of the reference, as skilled artisans, that combined testing of molecular markers with FOBT will yield any particular result with respect to the accuracy of diagnostic screening for colorectal cancer. Kutzner provides no more than a guess that the approach *might be* successful. Kutzner's express statement that results presented "**do not allow any conclusion to be formed"** contradicts the rationale offered by Examiner, and shows that an expectation of enhanced accuracy of diagnosis of colorectal cancer could not be deemed a *reasonable* expectation from the teachings of these references.

Regarding a threshold value for hemoglobin, the Examiner points to Yee as setting a 10 ng/ml of hemoglobin in blood as a threshold value. However, without acquiescing to the Examiner's characterization of Yee, like Kikuiri, Yee makes no mention of analyzing multiple portions of a fecal specimen, nor of partitioning a fecal sample for combining different types of testing. Thus, while Yee may disclose a fecal test reciting a 10 ng/ml for hemoglobin, Yee does not cure the deficiencies of Kutzner, Shuber, and Kikuiri with respect to instant claims 87 and 89-94, as the combination of these references does not teach or suggest obtaining a pair of portions of a fecal sample from a human subject without freezing using a method comprising collection of the fecal sample at home by the human subject by defecation directly into a sealable collection vessel, wherein a portion of the fecal sample is removed to a separate container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, nor of testing the removed portion of the fecal sample for a concentration of hemoglobin and testing DNA from the remaining portion of the same fecal sample for DNA from the human subject that has an epigenetic modification.

For the reasons set forth above, Applicant submits that the combination of Kutzner, Shuber, Kikuiri, and Yee does not establish *prima facie* obviousness of claims 87 and 89-94 as amended for at least the reason that the combination of references does not teach or suggest all the elements of the claims, and that the modifications urged by the Examiner are not supported by any reasonable expectation of success. Applicant therefore respectfully requests that the rejection be withdrawn.

IV. Claims 87, 95-100 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Kutzner in view of Shuber as applied to claim 87, and further in view of either Kim or Van Engeland.

The non-obviousness of claim 87 over Kutzner and Shuber as applied to claim 87 is discussed above and is applied here. Claim 95 is canceled in the present amendment and remaining claims 96-100 depend from claim 87 and thereby comprise all the same elements of claim 87.

As acknowledged by the Examiner in ¶29 of the Office Action, Kutzer in view of Shuber does not teach limitations such as specific non-invasive molecular biomarker detection with various epigenetic biomarkers. *e.g.,* hypermethylation level.

The Examiner points to Kim or Van Engeland as allegedly teaching colorectal cancer epigenetic markers. (Office Action, ¶¶30-31). However, neither Kim nor Van Engeland cure the deficiencies of Kutzner and Shuber discussed above. Further, Van Engeland is not prior art to the instant application.

Regarding Van Engeland, Applicant notes that the present application claims priority to U.S. Provisional Application No. 61/149,581, filed on February 3, 2009, which is less than one year from the publication date of the Van Engeland reference. Van Engeland therefore could be prior art to the present application only under pre-AIA 35 U.S.C. § 102(a).

Applicant has attached hereto an Inventor's Declaration Under 37 C.P.R. § 1.132 by inventor of the present invention Joost Louwagie ("Louwagie Decl."), which was originally filed with the Office in Appl. Ser. No., 13/147,570, to which the instant application claims priority as a continuation of U.S. Application No. 15/010,436, filed January 29, 2016, which is a continuation of U.S. Application No. 13/147,570. Inventor Louwagie states in ¶3 of the

Declaration that "In so far as the '219 Int'l Published App [WO 2008/084219] is asserted to disclose methods of determining a predisposition to, or the incidence of, colorectal cancer in a fecal sample from a human patient, comprising detecting methylation of one or more CpG dinucleotide motifs in the promoter of NDRG4 DNA contained within the fecal sample, these methods were invented by me and no other." Thus, the Louwagie Decl. established that the Van Engeland publication cited by the Examiner does not disclose invention "by another," as required under pre-AIA 35 U.S.C. § 102(a), and therefore does not qualify as prior art to the present application under § 102(a).

Examiner points to Kim as allegedly teaching "colorectal cancer epigenetic biomarkers are used for the early detection along of[sic] colorectal cancer with fecal-occult blood test (FOB)" pointing to page 686, Table 1, particularly column 1 bridging column 2 of page 689. (Office Action, ¶30). Applicant respectfully disagrees with the Examiner's characterization of Kim, as Kim makes no mention of testing epigenetic biomarkers along with FOB on the same fecal specimen. Rather, Kim is a review article that catalogues the "noninvasive molecular biomarkers that are currently in clinical settings and have the potential for use as CRC biomarkers" as of the time Kim was published in 2008 (Kim, Abstract). While Kim references the NDRG4 marker, nothing in Kim teaches or suggests combining detection of NDRG4 (or any other colorectal cancer epigenetic biomarkers) along with fecal-occult blood testing on separately stabilized portions of the same freshly collected fecal sample, as recited in Applicant's claims.

Thus, Kim does not cure the deficiencies of Kutzner and Shuber with respect to instant claims 87 and 96-100, as the combination of these references does not teach or suggest obtaining a pair of portions of a fecal sample from a human subject without freezing using a method comprising collection of the fecal sample at home by the human subject by defecation directly into a sealable collection vessel, wherein a portion of the fecal sample is removed to a separate container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, nor testing the removed portion of the fecal sample for a concentration of hemoglobin and testing DNA from the remaining portion of the same fecal sample for DNA

from the human subject that has an epigenetic modification.

The Examiner further asserts that "In view of the teaching of the combined cited art such as Kutzner and Kim, which teaches a desire for accurate detection of early colorectal cancer with usages of various known molecular DNA biomarkers in addition with the FOBT, one of ordinary skilled artisans would have been motivated have[sic] employed admittedly known methods so to detect not only occult blood (hemoglobin) but known and useful gene targets, and to also determine whether the target sequences such as NDRG4 also demonstrate hypermethylation, be it in a gene of interest or a promoter of same" (Office Action, ¶37). However, teaching "a desire for accurate detection of early colorectal cancer" is not a teaching that the combination can be expected to successfully provide the desired result. As noted above, Kutzner clearly states that their "retrospective data *do not allow any conclusion* on the potential of this approach in diagnostic screening" and that prospective comparative studies that include very high number of individuals is required to prove the value of the combined application of molecular testing and FOBT. (Kutzner, p40, paragraph bridging columns 1-2). Thus, it is not obvious even to the authors of the reference, as skilled artisans, that combined testing of molecular markers with FOBT will yield any particular result with respect to the accuracy of diagnostic screening for colorectal cancer. Kutzner provides no more than a guess that the approach *might be* successful. Kutzner's express statement that results presented "**do not allow any conclusion to be formed"** contradicts the rationale offered by Examiner, and shows that an expectation of enhanced accuracy of diagnosis of colorectal cancer could not be deemed a *reasonable* expectation from the teachings of these references. It is, at best, a desired outcome.

For the reasons set forth above, Applicant submits that the combination of Kutzner, Shuber, and Kim does not establish *prima facie* obviousness of amended claim 87 and 96-100 for at least the reasons that the combination of references does not teach or suggest all the elements of the claims, and that the modifications urged by the Examiner are not suggested by the cited art or other evidence, nor are they supported by any reasonable expectation of success. Applicant therefore respectfully requests that the rejection be withdrawn.

Applicant's alleged admissions

In addition to the references discussed above, the Examiner characterized a number of

items from Applicant's Specification as applicant admissions. See, e.g., Office Action at ¶¶ 32, 32, 33, and 37. While the alleged applicant admissions are not enumerated or discussed here, the absence of discussion in this response is not to be taken as a concession that any information from Applicant's Specification is conceded by the Applicant to be prior art to the instant claims, nor that the Examiner's characterization of any such alleged admission is correct.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that all grounds for rejection have been addressed and Applicant's claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned at (608) 662-1277.

Dated: November 29, 2021

/Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	:	13/147,570
Applicant	:	Joost Louwagie
Filed	:	March 12, 2012
Title	:	METHODS FOR DETECTING COLORECTAL CANCER
TC/A.U.	:	1634
Examiner	:	Bradley L. Sisson
Docket No.	:	5545-00036
Conf. No.	:	4272

INVENTOR'S DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

I, Joost Louwagie, hereby declare and state that:

1. I am the sole inventor of the methods of determining a predisposition to, or the incidence of, colorectal cancer in a fecal sample from a human patient, comprising (a) detecting human blood in the fecal sample; and (b) detecting methylation of one or more CpG dinucleotide motifs in the promoter of NDRG4 in DNA contained within the fecal sample, as disclosed and presently claimed in the above-captioned U.S. Patent Application No. 13/147,570 (hereinafter "the '570 Non-Provisional Application"), filed on March 12, 2012, which is the U.S. National Stage of International Application PCT/GB2010/000180, filed on February 3, 2010.

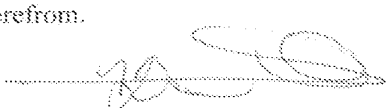
2. I am the sole inventor of the methods of determining a predisposition to, or the incidence of, colorectal cancer in a fecal sample from a human patient, comprising (a) detecting human blood in the fecal sample; and (b) detecting methylation of one or more CpG dinucleotide motifs in the promoter of NDRG4 in DNA contained within the fecal sample as disclosed and claimed in U.S. Provisional Application No. 61/149,581, filed on February 3, 2009 (hereinafter "the '581 Provisional Application") to which the '570 Non-Provisional Application claims the benefit of priority.

3. I am a co-inventor of the subject matter disclosed in International Application PCT/GB2008/000056, which lists as inventors Manon Van Engeland, Manon Adrian De Bruine, Arjan Griffioen, myself, and Katja Bierau and was published as WO 2008/084219 on July 17, 2008 (hereinafter the '219 Int'l Published App"). In so far as the '219 Int'l Published App is asserted to disclose methods of determining a predisposition to, or the incidence of, colorectal cancer in a fecal sample from a human patient, comprising detecting methylation of one or more CpG dinucleotide motifs in the promoter of NDRG4 in DNA contained within the fecal sample, these methods were invented by me and no other.

4. The subject matter disclosed in the '570 Non-Provisional Application and the '581 Provisional Application and related to methods of determining a predisposition to, or the incidence of, colorectal cancer in a fecal sample from a human patient, comprising (a) detecting human blood in the fecal sample; and (b) detecting methylation of one or more CpG dinucleotide motifs in the promoter of NDRG4 in DNA contained within the fecal sample was invented solely by me and no others.

5. The listed co-inventors of the '219 Int'l Published App, other than myself, namely Manon Van Engeland, Manon Adrian De Bruine, Arjan Griffioen, and Katja Bierau, did not make an inventive contribution to the subject matter claimed in the '570 Non-Provisional Application and the '581 Provisional Application and therefore are not co-inventors of the subject matter claimed in the '570 Non-Provisional Application and the '581 Provisional Application.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

By  Date: July 7, 2016

Joost Louwagie

Electronic Patent Application Fee Transmittal

Application Number:	15634607
Filing Date:	27-Jun-2017
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Filer:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	1252	1	640	640
Miscellaneous:				
		Total in USD ($)		**640**

Electronic Acknowledgement Receipt	
EFS ID:	44384222
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow/Stephanie Filandrinos
Filer Authorized By:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	29-NOV-2021
Filing Date:	27-JUN-2017
Time Stamp:	16:53:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$640
RAM confirmation Number	E2021ASG53382438
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2021-11-29_35239-304_ROAb.pdf	1736148 dcda44c903a74cb12e7cc2b674b5c565e503cbf4	yes	16
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	4	
	Applicant Arguments/Remarks Made in an Amendment		5	16	
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38400 e9f4697cde42c3633f1fcb9efed1a625d5659072	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1774548		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/634,607	Filing Date 06/27/2017	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	11/29/2021	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 14	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/DEIDRE C KING-WOODWARD/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Joost		Louwagie	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Dornach	Country of Residence	CH

Mailing Address of Inventor:

Address 1	441 Charmany Drive		
Address 2			
City	Madison	**State/Province**	WI
Postal Code	53719	**Country**	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	72960		
Email Address	docketing@casimirjones.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS FOR DETECTING COLORECTAL CANCER		
Attorney Docket Number	EXCT-35239/US-4/CON	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	72960		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	15010436	2016-01-29

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15010436	Continuation of	13147570	2012-03-12
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13147570	a 371 of international	PCT/GB2010/000180	2010-02-03
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/GB2010/000180	Claims benefit of provisional	61149581	2009-02-03

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name: ~~Exact Sciences Development Company, LLC~~ Exact Sciences Corporation

Mailing Address Information For Applicant:

Address 1	5505 Endeavor Lane		
Address 2			
City	Madison	**State/Province**	WI
Country	US	Postal Code	53719
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Mary Ann D. Brow/			Date (YYYY-MM-DD)	2019-05-23
First Name	Mary Ann D.	Last Name	Brow	Registration Number	42363

Additional Signature may be generated within this form by selecting the Add button.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	EXCT-35239/US-4/CON
	Application Number	
Title of Invention	METHODS FOR DETECTING COLORECTAL CANCER	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: R46C.REQ
Document Description: Request under 37 CFR 1.46(c) to correct, update or change the applicant.

PTO/AIA/41 (04-15)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
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REQUEST TO CORRECT OR UPDATE THE NAME OF THE APPLICANT UNDER 37 CFR 1.46(c)(1), OR CHANGE THE APPLICANT UNDER 37 CFR 1.46(c)(2)

(FOR USE ONLY IN APPLICATIONS FILED ON OR AFTER SEPTEMBER 16, 2012)

Application Number	15/634,607
Filing Date	27-Jun-2017
First Named Inventor	JOOST
Art Unit	
Examiner Name	
Practitioner Docket Number	EXCTD-35239.304

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Applicant hereby **requests that the name of the applicant be corrected or updated under 37 CFR 1.46(c)(1), or that the applicant be changed under 37 CFR 1.46(c)(2),** in the above-identified application. **Requests under 37 CFR 1.46(c)(1) or (c)(2) cannot be submitted after payment of the issue fee or if the application has been patented.**

Please check the applicable box(es) below.

☒ 1. This request is to **correct or update the name of the applicant** (**under 37 CFR 1.46(c)(1)**) and includes:

☒ An application data sheet (ADS) in accordance with 37 CFR 1.76(c) with the corrected or updated information shown with markings (*e.g.*, underlining for insertions, strikethrough for deletions). A Corrected Web-based ADS may be used.

Note: Requests under 37 CFR 1.46(c)(1) may be filed to correct typographical errors in the name of the § 1.46 applicant, or for updating the name of the § 1.46 applicant (*i.e.*, where there is no change in the applicant itself but just in the applicant's name). See the Manual of Patent Examining Procedure (MPEP) section 605.01.

☐ 2. This request is to **change the applicant** (**under 37 CFR 1.46(c)(2)**) and includes:

☐ An application data sheet (ADS) in accordance with 37 CFR 1.76(c) that identifies the changes with proper markings (underlining for insertions and strikethrough for deletions). A Corrected Web-based ADS may be used.

☐ A Statement Under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent). See MPEP 325.

I am the

☐ applicant* ☒ attorney or agent of record Registration number 42363 ☐ attorney or agent acting under 37 CFR 1.34 Registration number _____

Signature /Mary Ann D. Brow/
Typed or printed name Mary Ann D. Brow
Date 2022-03-15

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. *Juristic entities must be represented by a patent practitioner (See 37 CFR 1.31, applicable to any paper filed on or after September 16, 2012 that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below**.

☒ ** Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.36. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	45229344
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow/Jeremiah Gile
Filer Authorized By:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	15-MAR-2022
Filing Date:	27-JUN-2017
Time Stamp:	15:50:43
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	2022-03-15_35239-304_ADS-Marked-Up.pdf	141432 6a9ef364d279c6ed909c5225623d7c6404d9dfae	no	8

Warnings:

Information:					
This is not an USPTO supplied ADS fillable form					
2	Request under 37CFR1.46(c) to correct, update, change applicant	2022-03-15_35239-304_CorrApplForm.pdf	202369 37b1e4a5f7f1d0918817a66a5a5ec52e24964e64	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	343801	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 03/25/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
SISSON, BRADLEY L	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
03/25/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@CASIMIRJONES.COM
Genomic_docketing@cardinal-ip.com

Office Action Summary	Application No. 15/634,607	Applicant(s) Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 11/29/2021.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 82,84,87,89-94 and 96-100 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 82,84,87,89-94 and 96-100 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

Claim Interpretation

2. Attention is directed to MPEP 904.01 [R-08.2012].

> The breadth of the claims in the application should always be carefully noted; that is, the examiner should be fully aware of what the claims do not call for, as well as what they do require. During patent examination, the claims are given the broadest reasonable interpretation consistent with the specification. See *In re Morris*, 127 F.3d 1048, 44 USPQ2d 1023 (Fed. Cir. 1997). See MPEP § 2111 - § 2116.01 for case law pertinent to claim analysis.

3. It is noted with particularity that narrowing limitations found in the specification cannot be inferred in the claims where the elements not set forth in the claims are linchpin of patentability. *In re Philips Industries v. State Stove & Mfg. Co, Inc*., 186 USPQ 458 (CA6 1975). While the claims are to be interpreted in light of the specification, it does not follow that limitations from the specification may be read into the claims. On the contrary, claims must be interpreted as broadly as their terms reasonably allow. See *Ex parte Oetiker,* 23 USPQ2d 1641 (BPAI, 1992). In added support of this position, attention is directed to MPEP 2111 [R-11.2013], where, citing *In re Prater*, 415 F.2d 1393, 1404-05, 162 USPQ 541, 550-51 (CCPA 1969), is stated:

> The court explained that "reading a claim in light of the specification, to thereby interpret limitations explicitly recited in the claim, is a quite different thing from 'reading limitations of the specification into a claim,' to thereby narrow the scope of the claim by implicitly adding disclosed limitations which have no express basis in the claim." The

court found that applicant was advocating the latter, i.e., the impermissible importation of subject matter from the specification into the claim.

4. Additionally, attention is directed to MPEP 2111.01 [R-07.2015], wherein is stated:

II. IT IS IMPROPER TO IMPORT CLAIM LIMITATIONS FROM THE SPECIFICATION

"Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim. For example, a particular embodiment appearing in the written description may not be read into a claim when the claim language is broader than the embodiment." *Superguide Corp. v. DirecTV Enterprises, Inc.*, 358 F.3d 870, 875, 69 USPQ2d 1865, 1868 (Fed. Cir. 2004).

5. Attention is also directed to MPEP 2111.02 II. As stated herein:

II. PREAMBLE STATEMENTS RECITING PURPOSE OR INTENDED USE
The claim preamble must be read in the context of the entire claim. The determination of whether preamble recitations are structural limitations or mere statements of purpose or use "can be resolved only on review of the entirety of the [record] to gain an understanding of what the inventors actually invented and intended to encompass by the claim." *Corning Glass Works,* 868 F.2d at 1257, 9 USPQ2d at 1966. **If the body of a claim fully and intrinsically sets forth all of the limitations of the claimed invention, and the preamble merely states, for example, the purpose or intended use of the invention, rather than any distinct definition of any of the claimed invention's limitations, then the preamble is not considered a limitation and is of no significance to claim construction.** *Pitney Bowes, Inc. v. Hewlett-Packard Co.,* 182 F.3d 1298, 1305, 51 USPQ2d 1161, 1165 (Fed. Cir. 1999). See also *Rowe v. Dror,* 112 F.3d 473, 478, 42 USPQ2d 1550, 1553 (Fed. Cir. 1997) ("where a patentee defines a structurally complete invention in the claim body and uses the preamble only to state a purpose or intended use for the invention, the preamble is not a claim limitation")... (Emphasis added)

6. Attention is directed to MPEP 2111. As stated therein:

During patent examination, the pending claims must be "given their broadest reasonable interpretation consistent with the specification." The Federal Circuit's *en banc* decision in *Phillips v. AWH Corp.*, 415 F.3d 1303, 1316, 75 USPQ2d 1321, 1329 (Fed. Cir. 2005) expressly recognized that the USPTO employs the "**broadest reasonable interpretation**" standard:

> The Patent and Trademark Office ("PTO") determines the scope of claims in patent applications not solely on the basis of the claim language, but upon giving claims their broadest reasonable construction "in light of the specification as it would be interpreted by one of ordinary skill in the art." *In re Am. Acad. of Sci. Tech. Ctr.*, 367 F.3d 1359, 1364[, 70 USPQ2d 1827, 1830] (Fed. Cir. 2004). Indeed, the rules of the PTO require that application claims must "conform to the invention as set forth in the remainder of the specification and the terms and phrases used in the claims must find clear support or antecedent basis in the description so that the meaning of the terms in the claims may be ascertainable by reference to the description." 37 CFR 1.75(d)(1). (Emphasis added).

Claim Rejections - 35 USC § 103

7. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

8. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Attention is directed to the decision in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007):

> When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

10. It is further noted that prior art is not limited to the four corners of the documentary prior art being applied. Prior art includes both the specialized understanding of one of ordinary skill in the art, and the common understanding of the layman. It includes "background knowledge possessed by a person having ordinary skill in the art. . . [A] court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR* at 1396.

11. Suggestion, teaching or motivation does not have to be explicit and "may be found in any number of sources, including common knowledge, the prior art as a whole or the nature of the problem itself'" *Pfizer, Inc. v. Apotex, Inc.* 480 F.3d 1348, 82 USPQ2d 1321 (Fed. Cir. 2007) citing *Dystar Textilfarben GMBH v. C. H. Patrick Co.*, 464 F.3d 1356 (Fed. Cir. 2006).

Admissions as Prior Art.

12. Attention is directed to MPEP 706.02 [R-07.2015]:

> **III. RELIANCE ON ADMITTED PRIOR ART IN SUPPORT OF REJECTION**
> A statement by an applicant in the specification or made during prosecution identifying the work of another as "prior art" is an admission which can be relied upon for both anticipation and obviousness determinations, regardless of whether the admitted prior art would otherwise qualify as prior art under the statutory categories of 35 U.S.C. 102. *Riverwood Int'l Corp. v. R.A. Jones & Co.*, 324 F.3d 1346, 1354, 66 USPQ2d 1331, 1337 (Fed. Cir. 2003); *Constant v. Advanced Micro-Devices Inc.*, 848 F.2d 1560, 1570, 7 USPQ2d 1057, 1063 (Fed. Cir. 1988). See MPEP § 2129 for discussion on admissions as prior art. Where the admitted prior art anticipates the claim but does not qualify as prior art under any of the paragraphs of 35 U.S.C. 102, the claim may be rejected as being anticipated by the admitted prior art without citing to 35 U.S.C. 102.

13. Attention is directed to MPEP 2129 [R-08.2012], Admissions as Prior Art, which states in part:

> **I. ADMISSIONS BY APPLICANT CONSTITUTE PRIOR ART**

> A statement by an applicant in the specification or made during prosecution identifying the work of another as "prior art" is an admission **>which can be relied upon for both anticipation and obviousness determinations, regardless of whether the admitted prior art would otherwise qualify as prior art under the statutory categories of 35 U.S.C. 102. *Riverwood Int'l Corp. v. R.A. Jones & Co.*, 324 F.3d 1346, 1354, 66 USPQ2d 1331, 1337 (Fed. Cir. 2003); *Constant v. Advanced Micro-Devices Inc.*, 848 F.2d 1560, 1570, 7 USPQ2d 1057, 1063 (Fed. Cir. 1988).

Holding and Rationale

14. Claims 82, 84, 87, 96, and 97 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41).

15. Wang et al., teach of a device that can be used for collecting a fecal sample, mixing said sample with a "preserving fluid", that the sample is not frozen (wet), yet is transported to a testing lab. As stated in the abstract:

> A device for quantitively **collecting, preserving, storing and mailing** a fresh and **wet** specimen of **fecal** or other biological matter for later analysis comprises a simple tubular vessel double sealed at one end by a machine manipulable plug having breakable hollow nib and cover, and that is engaged at the opposite end by a machine manipulable stopper from which a specimen carrying stick axially projects into the vessel through an internal sealable septum spanning a median section of the vessel. The cross-sectional profile of the stick is shaped to form a widened shoulder carrying a resilient washer. The shoulder matingly engages a correspondingly shaped passageway in the septum when the stopper engages the vessel which restricts the amount of specimen passing therethrough. The amount of specimen and **preserving fluid** are quantitatively balanced and remain so until part or all of the fluid is extracted for analysis either manually by breaking of the sealing nib or automatedly by machine removal of the plug or stopper. Collection by a patient or unskilled person is enhanced by providing the tubular vessel as part of a sample collection and return kit which further contains a disposable paper catch web which is made to be temporarily secured to a toilet droopingly spanning the seat to catch the feces for sampling. The kit also provides a sealable plastic bag and a crush resistant mailing

> carton. The above components of the kit are carried within a sterile bag until used. (Emphasis added)

16. Wang et al., at paragraph [0012], teaches:

> [0012] Collection by a patient or unskilled person is enhanced by providing the tubular vessel as part of a sampling kit which further contains a disposable paper catch web which is made to be temporarily secured to a toilet droopingly spanning the seat to catch the feces for sampling. The kit also provides a sealable plastic bag and a crush resistant mailing carton. The above components of the kit are carried within a sterile bag until used. (Emphasis added)

17. While Wang et al., teach collection of a sample, they have not been found to teach collecting a portion of a sample, or pairs of samples and sending these removed portion(s) to a lab for testing.

18. Liang et al., teach of a device and method for collecting and testing fecal samples. As disclosed in paragraph **[0112]**, one can collect multiple samples via a "sampling wand", which is then inserted into a test apparatus, which is subsequently transported to a testing laboratory. As disclosed therein:

> **[0112]** An apparatus as illustrated in FIGS. 3A-3 G or 5A-B, and containing appropriate assay buffer in the sample-receiving chamber, is provided to a user. The user collects his/her feces, for example on a flushable, paper sheet secured across the seat of a toilet. The user removes the cap and the sampling wand from the rapid test apparatus, and inserts the sampling wand into the fecal sample, for example multiple times in order to obtain sample from different portions of the sample. The sampling wand (member)/cap portion is then reinserted into the test apparatus, which causes insertion of the sampling wand into the **buffer** within the sample-receiving chamber of apparatus. The cap is tightened securely and the apparatus is gently shaken to solubilize the fecal sample to obtain a liquid suspension of buffer and the biological sample. The test apparatus is then transported, for example, via first class mail, to a laboratory. (Emphasis added)

19. It would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the method and device of Wang et al., so to include the device of Liang et al.,

for to do so would capitalize on the fecal sample being accessible on the covered toilet and collection of multiple samples that can be sent independently to the testing facility for analysis.

20. Liang et al., teach of various assays that can be performed. As seen in "Example 1" one can use the device for testing of fecal occult blood. However, Liang et al., has not been found to teach concentrations of hemoglobin that can be detected.

21. Neither Wang et al., nor Liang et al., have been found to teach testing DNA for epigenetic modifications.

22. Olek et al., disclose methods for diagnosing illnesses and predisposition to certain illnesses. As seen in paragraph [0003]:

> In contrast to classical mutations, **DNA methylation** involves a mechanism that describes a base substitution without modifying the coding function of a gene. This interplay between **epigenetic modification** and classical mutations plays an important role in tumorigenesis. For example, **focal hypermethylation and generalized genomic demethylation are features of many different tumor types**. It is assumed that tumorigenesis and tumor progression are caused, first of all, by **hypermethylation** of induced mutation events, and secondly, by the turning off of genes which control cellular proliferation and/or by the induced reactivation of genes, which are [normally] used only for embryological development, via demethylation. (Emphasis added)

23. Olek et al., in paragraph [0029], teach that genomic DNA can be isolated from stool samples. As stated therein:

> **[0029] The genomic DNA to be analyzed is obtained preferably from the usual sources for DNA, such as**, e.g., cell lines, blood, sputum, **stool**, urine, cerebrospinal fluid, tissue embedded in paraffin, for example, tissue from eyes, intestine, kidney, brain, heart, prostate, lung, breast or liver, histological slides and all other possible combinations thereof. (Emphasis added)

24. The aspect of one testing DNA derived from a fecal sample so to determine if there is hypermethylation present is deemed to fairly suggest limitations of claims 96 and 97.

25. While Olek et al., teach performing tests on stool samples whereby one detects DNA sequence, they have not been found to teach testing for hemoglobin.

26. Kutzner et al., disclose method for the detection of colorectal tumors using a combination of tests- one which looks for a component of blood, and one which is based on nucleic acid analysis, e.g., PCR.

27. A seen therein, the faecal occult blood test was "a standard guaiac test", which is not an immunological test, deemed deficient, and does not seek to determine the amount of hemoglobin present.

28. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the methods and related devices of Wang et al., and Liang et al., so to include the testing of fecal samples for hemoglobin, hypermethylation/aberrant methylation and nucleic acid analysis, e.g., assaying for tumor genes. As evidenced above, Kutzner et al., teaches performing different types of assays on the fecal samples. By having different portions of a common fecal sample being produced and directed to the relevant lab, multiple assays can be performed in a more rapid pace, therein providing medical care in a more expeditious manner.

29. In view of the well-developed state of the art, said ordinary artisan would have had a most reasonable expectation of success.

30. In view of the above analysis and in the absence of convincing evidence to the contrary, claims 82, 84, 87, 96, and 97 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41).

31. Claims 89-94 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41), as applied to claims 82, 84, 87, 96, and 97, and further in view of US 2006/0188939 A1 (Gao) and Yeh (US 2008/0227298 A1).

32. See above for the basis of the rejection as it pertains to the disclosures of US 2006/0210448 A1 (Wang et al.), US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41).

33. While the prior art teaches detection of hemoglobin in a stool sample, they have not been found to teach that one can employ "immunochemical detection" (claim 89), nor disclose what concentration of hemoglobin detected is considered to be positive.

34. Gao teaches methods for detecting analytes in a fecal sample. As seen in paragraph [0041], such includes an immunoassay for fecal occult blood. Such a showing is deemed to fairly suggest limitation of claim 89.

35. While Gao teaches detecting hemoglobin in a fecal sample, Gao has not been found to teach the concentration of hemoglobin that can be detected.

36. Yee et al., paragraph [0041], teach setting a 10 ng/ml of hemoglobin in blood as a threshold value. Such a showing is deemed to meet a limitation of claims 90-94.

37. Applicant, at page 2 of the as-filed disclosure, under "BACKGROUND OF THE INVENTION," teaches that various methods, including immunochemical assays for detecting human hemoglobin in fecal sample was known in the art. As stated therein:

> Fecal occult blood tests fall primarily into two categories, tests based on the use of chromogenic chemical reagents such as gum guaiac and immunochemical tests. The chemically based guaiac methods determine the presence of occult blood by the detection of the perioxidase activity of the hemoglobin in the blood present in the faecal sample. They require catalysis of peroxide into oxygen and water, and the subsequent oxidation of a colorless dye (most often into a colored form). However, peroxidase activity is also found in meats and vegetables. In order to produce accurate results, these tests require restriction of the intake of certain foods, drugs, vitamins, and other substances prior to and during the sample collection period. The sensitivity of the most commonly used guaiac FOBT (Hemoccult) is approximately 50%. Despite a specificity of 98%, the positive predictive value for FOBT is low. Methods of detecting occult blood based on porphyrin (heme and protpoporphyrin IX) analysis or immunologic tests using anti-hemoglobin antibodies improve on these results**. Immunochemical tests (FIT or iFOBT) that use anti- hemoglobin antibodies specific for human blood in extracts from stool do not require dietary restrictions;** however, they are more complicated and more expensive than peroxidase-based tests. (Emphasis added).

38. It would have also been obvious to an ordinary artisan to have employed distinct portions from a subject's fecal sample in testing lab, as exemplified by Kutzner et al., to detect multiple signals indicative of colorectal cancer or tumor, e.g., an inclusion of detecting occult blood (hemoglobin) at various desired contractions based on known threshold levels. Said ordinary artisan would have been motived to have used immunochemicals/immunoassay for the detection of fecal hemoglobin as such methods and means of operation were clearly well known, thereby imparting a most reasonable expectation of success (Gao et al.). In light of the significant interest in testing patients' samples for such medically-significant target molecules, said ordinary artisan would have been amply motivated.

39. In view of the above analysis and in the absence of convincing evidence to the contrary, claims 89-94 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41), as applied to claims 82, 84, 87, 96, and 97, and further in view of US 2006/0188939 A1 (Gao) and Yeh (US 2008/0227298 A1).

40. Claims 98-100 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41), as applied to claims 82, 84, 87, 96, and 97, and further in view of either Kim et al. (BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008) or WO 2008/084219 A1 (Van England et al.).

41. See above for the bass of the rejection as it pertains to the disclosures of US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41)

42. Neither Wang et al., Liang et al., Olek et al., nor Kutzner et al., have been found to disclose the genes of claims 98 and 99, nor disclose the use of modifying DNA with bisulfite ions (claim 100).

43. Kim teaches colorectal cancer epigenetic biomarkers are used for the early detection of colorectal cancer with fecal-occult blood test (FOB). See page 686, Table 1, particularly column 1 bridging column 2 of page 689.

44. Applicant, page 5 of the disclosure, teaches that methods for detection of colorectal carcinoma based upon determining methylation of a number of different genes, including panels of genes, are disclosed in WO 2008/084219 A1 (Van England et al.).

> Van England relates to detection of colorectal cancer based upon determining methylation of a number of different genes, including panels of genes. The elected marker NDRG4 is also taught in Van England et al. (claims 1 and 2).

45. Applicant, at page 21, last paragraph, bridging to page 22 of the as-filed disclosure admits that numerous methods for assessing methylation are known in the art, including methods that comprise polymerase chain reaction (PCR), which is recognized as a form of amplifying DNA. Also admitted to therein is the use of chemical reagents so to modify the DNA prior to amplification was also well known in the art. As admitted to therein:

> **Determination of the methylation status may be achieved through any suitable means. Suitable examples include bisulphite genomic sequencing and/or by methylation specific PCR. Various techniques for assessing methylation status are known in the art and can be used in conjunction with the present invention**: sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR(McMS-PCR), MLPA with or without bisulphite treatment, QAMA (Zeschnigk et al, 2004), MSRE-PCR (Melnikov et al, 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulphite conversion-specific methylation-specific PCR (BS-MSP)(Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence diferences in PCR products of sodium bisulphite - treated DNA), methylation-sensitive single-nucleotide primer extension conformation(MS-SNuPE), methylation- sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulphite restriction analysis (McCOBRA)(Akey et al., 2002), PyroMethA, HeavyMethyl (Cottrell et al. 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques for DNA methylation analysis is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264, Nature Reviews, 2003, Vol.3, 253-266; Oral Oncology, 2006, Vol. 42, 5- 13, which references are incorporated herein in their entirety. (Emphasis added)

46. Applicant, at page 24, third paragraph, admits that methods for performing MSP, wherein one employs primer pairs designed to distinguish methylated DNA from unmethylated DNA, was known in the art. As admitted to therein:

> In the MSP approach, DNA may be amplified using primer pairs designed to distinguish methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulphite treatment (**Herman et al., 1996; and WO 97/46705**). For example, bisulphite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. (Emphasis added)

47. The above showing is deemed to meet a limitation of claim 100.

48. Applicant, at page 26, last paragraph, bridging to page 27, of the as-filed disclosure further asserts:

> Whilst PCR is the preferred nucleic acid amplification technique, other amplification techniques may also be utilised to detect the methylation status of the concerned gene. Such amplification techniques are well known in the art, and include methods such as NASBA (Compton, 1991), 3SR (Fahy et al., 1991) and Transcription Mediated Amplification (TMA). Other suitable amplification methods include the ligase chain reaction (LCR) (Barringer et al, 1990), selective amplification of target polynucleotide sequences (US Patent No. 6,410,276), consensus sequence primed polymerase chain reaction (US Patent No 4,437,975), arbitrarily primed polymerase chain reaction (WO 90/06995), invader technology, strand displacement technology, and nick displacement amplification (WO 2004/067726). This list is not intended to be exhaustive; any nucleic acid amplification technique may be used provided the appropriate nucleic acid product is specifically amplified. Thus, these amplification techniques may be tied in to MSP and/or bisulphite sequencing techniques for example. (Emphasis added)

49. In view of the above showing, it would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the methods of Liang et al., Olek et al., Kutzner et al. in view of Shuber, whereby one was detecting known, informative genes, such as the elected NDG4, TFPI2, PHACTR3, SFRP2 and SOX17, whereby one was using different portions of a faecal sample for the immunoassay and the nucleic acid-based assay, e.g., hypermethylation detection assays. It would have also been obvious to said ordinary artisan to

have employed means of stabilizing the sample portions for to do so would have a direct an immediate effect of being able to accurately and reproducibly determine the level of hemoglobin present, as well as preserve any nucleic acid present in the cells, and therein enhancing the ability to obtain accurate results.

50. In view of the teaching of the combined cited art such as Kutzner and Kim, which teaches a desire for accurate detection of early colorectal cancer with usages of various known molecular DNA biomarkers in addition with the FOBT, one of ordinary skilled artisans would have been motivated have employed admittedly known methods so to detect not only occult blood (hemoglobin) but known and useful gene targets, and to also determine whether the target sequences such as NDRG4 also demonstrate hypermethylation, be it in a gene of interest or a promoter of same.

51. In view of the well-developed state of the art and significant interest in being able to detect cancer at an early stage via both immunochemistry and nucleic acid analysis, said ordinary artisan would have been both well motivated and would have had a most reasonable expectation of success.

52. In view of the above analysis and in the absence of convincing evidence to the contrary, claims 98-100 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over US 2006/0210448 A1 (Wang et al.) in view of US 2009/0004058 A1 (Liang et al.), US 2005/0064401 A1 (Olek et al.) and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41), as applied to claims 82, 84, 87, 96, and 97, and further in view of

either Kim et al. (BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008) or WO 2008/084219 A1 (Van England et al.).

Conclusion

53. Objections and/or rejections which appeared in the prior Office action and which have not been repeated hereinabove have been withdrawn.

54. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

55. A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

56. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Bradley L. Sisson whose telephone number is (571)272-0751. The examiner can normally be reached Monday to Thursday, from 6:30 AM to 5 PM..

57. Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is

encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

58. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

59. Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Bradley L. Sisson/
Primary Examiner, Art Unit 1634

Notice of References Cited	Application/Control No. 15/634,607	Applicant(s)/Patent Under Reexamination Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20060210448-A1	09-2006	Wang; Naishu	A61B10/0038	422/400
*	B	US-20090004058-A1	01-2009	Liang; Greg	B01L3/502	422/68.1
*	C	US-20080124714-A1	05-2008	Shuber; Anthony P.	C12Q1/6806	435/6.16
*	D	US-20060216830-A1	09-2006	Kikuiri; Hideki	B01L3/5082	436/165
*	E	US-20080227208-A1	09-2008	Yee; Hsiao-Ching	G01N33/72	436/66
*	F	US-20050064401-A1	03-2005	Olek, Alexander	C07K14/4703	435/6.12
*	G	US-20060188939-A1	08-2006	Gao; Ping	A61B10/0038	435/7.1
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
*	N	WO-2008084219-A1	07-2008	WO	Van England et al.	A61P35/04
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
*	U	Kutzner et al., "Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", Cancer Letters" 229 (2005) 33-41. (Year: 2005)
*	V	Kim et al., BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008. (Year: 2008)
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022			
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
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	86	÷	-	-	-	-	-			
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	93	÷	✓	✓	✓	✓	✓			
	94	÷	✓	✓	✓	✓	✓			

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022			
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	97	÷	✓	✓	✓	✓	✓			
	98	÷	✓	✓	✓	✓	✓			
	99	÷	✓	✓	✓	✓	✓			
	100	÷	✓	✓	✓	✓	✓			

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.
EAST	06/16/2021	B. L. S.
EAST	03/22/2022	B. L. S.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	128	DNA same "epigenetic modification" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/22 17:47
L2	1	DNA same "epigenetic modification" and (faecal fecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/22 17:47
L3	20	DNA same "epigenetic modification" and (faecal fecal stool) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/22 17:48
L4	21	DNA same "epigenetic modification" and (faecal fecal stool feces) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/22 17:48
L5	19	DNA same "epigenetic modification" and (dna "nucleic acid" chromosomal chromatin) same (faecal fecal stool feces) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/22 17:49
L6	0	"20050064401" and (hemoglobin haemoglobin)	US-PGPUB	OR	OFF	2022/03/22 18:07
L7	112	immunoassay same (hemoglobin haemoglobin) and @pd<"20090203"	US-PGPUB	OR	OFF	2022/03/22 18:33
L8	51	immunoassay with (hemoglobin haemoglobin) and @pd<"20090203"	US-PGPUB	OR	OFF	2022/03/22 18:33
L9	2	immunoassay with (hemoglobin haemoglobin) same (stool fecal faecal) and @pd<"20090203"	US-PGPUB	OR	OFF	2022/03/22 18:44
S1	53	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:50

S2	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same "ng/ml" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 10:51
S3	9	(hemoglobin haemoglobin) with concentration same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:18
S4	260	DNA same ((stool fecal faecal) adj sample) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 11:35
S5	2	"20080227208"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:34
S6	1	"20080227208" and "10 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:35
S7	11	(hemoglobin haemoglobin) with "5 ng/ml"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/06/23 13:38
S8	0	NDGR4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:50
S9	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:50
S10	6	NDRG4 same epigenetic and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 13:58
S11	24	"2006113770"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	OFF	2020/01/14 14:02

			DERWENT; IBM_TDB			
S12	4	"2006113770" and NDRG4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:11
S13	6	NDRG4 same (epigenetic methylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S14	6	NDRG4 same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:19
S15	0	"N-myc downstream regulated gene 4" same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S16	0	"N-myc downstream regulated gene 4" and (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:24
S17	0	"N-myc downstream regulated gene 4" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:25
S18	1	"20060019256" and uptg	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 14:55
S19	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/14 15:35
S20	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:07

S21	3	fecal same (antibody antibodies immunoassay) same (DNA RNA polynucleotide "nucleic acid" gene) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 08:26
S22	2	(cancer carcinoma) same (PHACTR3 NDRG4 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFRP2 CDO1) same (hyridization hybridizes hybridize) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:40
S23	1	"200483399"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:42
S24	0	"200483399A2"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/01/15 10:43
S25	0	"200483399A2"	EPO	OR	OFF	2020/01/15 11:11
S26	0	"200483399"	EPO	OR	OFF	2020/01/15 11:11
S27	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 18:14
S28	38	NDRG4 and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/08/04 19:10
S29	15	hemoglobin with (stool fecal) with concentration and @pd<"20090203"	USPAT	OR	OFF	2021/06/15 10:13
S30	32	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and (antibody antibodies immunoassay immunological) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:33
S31	16	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:33

S32	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and "ng/ml" and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 11:37
S33	1,048	(freeze freezing frozen) same (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:05
S34	399	(freeze freezing frozen) with (stool fecal faecal) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:05
S35	236	(freeze freezing frozen) with (stool fecal faecal) with (sample fraction portion historical) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:07
S36	14	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion historical) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:07
S37	4	(immunoassay elisa) with (stool fecal faecal) with (fresh "non-frozen") and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 12:31
S38	1	"7832219".FMID.	US-PGPUB; USPAT; FPRS; JPO	OR	OFF	2021/06/16 12:32
S39	4	"7832219"	USPAT	OR	ON	2021/06/16 12:33
S40	2	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion historical) same buffer and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 13:30
S41	5	(freeze freezing frozen) same (stool fecal faecal) with (fraction portion historical) same buffer and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2021/06/16 13:33
S42	230	"5741650"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	ON	2021/06/16 13:38

			DERWENT; IBM_TDB			
S43	114	"5741650"	USPAT	OR	ON	2021/06/16 13:38
S44	63	"5741650" and frozen	USPAT	OR	ON	2021/06/16 13:40
S45	65	"5741650" and (freeze freezing frozen)	USPAT	OR	ON	2021/06/16 13:41
S46	3	(feces fecal faecal faeces) with buffer with (freeze freezing frozen) and @pd<"20090203"	USPAT	OR	ON	2021/06/16 13:51
S47	0	fecal with sample same (unfrozen "not frozen") and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/17 14:40
S49	85	fecal with sample same (frozen freezing) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/17 14:42
S50	12	fecal with sample same (sealable sealed seal) with (vessel container) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/17 14:57
S51	17	fecal with sample same (unfrozen "not frozen" freezing) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 15:56
S52	4	"20080124714"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 15:58
S53	6	fecal with sample same (sealable sealed seal) with (vessel container) same portion and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 16:18
S54	179	(stool fecal faecal) with sample with portion and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 16:52

S55	2	(stool fecal faecal) with sample with portion with (multiple duplicate) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/03/21 16:54
S56	69,546	((dna with (isolate extract extracted isolating)) (determine determining determins determined) with (hemoglobin haemoglobin) same (fecal faecal)) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 17:27
S57	1	((dna with (isolate extract extracted isolating)) and (determine determining determins determined) with (hemoglobin haemoglobin) same (fecal faecal)) and @pd<"20090203"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2022/03/21 17:28

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CONFIRMATION NO. 1874

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/634,607	06/27/2017	435	1634	EXCTD-35239.304

APPLICANTS
EXACT SCIENCES DEVELOPMENT COMPANY, LLC, Madison, WI;

INVENTORS
Joost Louwagie, Dornach, SWITZERLAND;

**** CONTINUING DATA ***************************
This application is a CON of 15/010,436 01/29/2016 ABN
which is a CON of 13/147,570 03/12/2012 ABN
which is a 371 of PCT/GB2010/000180 02/03/2010
which claims benefit of 61/149,581 02/03/2009

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
07/12/2017

Foreign Priority claimed	❑ Yes ☑ No	❑ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	❑ Yes ☑ No		SWITZERLAND	0	19	2
Verified and Acknowledged	/BRADLEY L SISSON/ Examiner's Signature	Initials				

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TITLE
FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

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15634607	CTFR	03/25/2022	EXCTD-35239.304
	892	03/25/2022	EXCTD-35239.304

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Doc Code: A.NE.AFCP
Document Description: After Final Consideration Pilot Program Request

PTO/SB/434 (05-13)

CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0

Practitioner Docket No.:	Application No.:	Filing Date:
EXCTD-35239.304	15/634,607	06/27/2017
First Named Inventor:	**Title:**	
Joost Louwagie	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD	

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.

1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (*e.g.*, a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c).
2. The above-identified application contains an outstanding final rejection.
3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect.
4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection.
5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response.
6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web).
7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.]
8. By filing this certification and request, applicant acknowledges the following:
   - Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0.
   - The examiner will verify that the AFCP 2.0 submission is compliant, *i.e.*, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions:
     - The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, by mailing an advisory action.
     - If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview.
       - The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate.
       - If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.

Signature	Date
/Mary Ann D. Brow/	2022-05-25
Name (Print/Typed) Mary Ann D. Brow	Practitioner Registration No. 42363

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.*

[✓] * Total of 1 forms are submitted.

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The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

AMENDMENT UNDER 37 C.F.R. §1.116 AFTER FINAL OFFICE COMMUNICATION MAILED 03/25/2022 AND REQUEST FOR AFTER-FINAL CONSIDERATION UNDER AFCP 2.0

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This communication is an After Final Amendment under 37 C.F.R. §1.116(b)(2), responsive to the Final Office Communication mailed 03/25/2022, with a response due on or before 06/25/2022, and is filed with a Request for After-Final Consideration. Applicants respectfully request entry of this amendment.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: **EXCTD-35239.304**. This paragraph is a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Amendments to the Claims are reflected in the Listing of Claims beginning on Page 2;

Remarks begin on Page 5.

AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior listings and versions of claims in the application:

1-81. (canceled)

82. (currently amended) A method of processing a freshly-collected fecal sample without freezing, the method comprising:

a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;

b) removing a portion of said fecal sample to a separate sealable container to produce a removed portion and a remaining portion of said fecal sample;

c) combining the removed portion in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and

d) stabilizing the remaining portion of the fecal sample by adding a buffer that maintains DNA integrity in a fecal sample to the remaining portion in the sealable collection vessel, and sealing the sealable collection vessel.

83. (canceled)

84. (currently amended) The method of claim 82, further comprising delivering the sealable container containing the removed portion and buffer and the sealable collection vessel containing the remaining portion of the fecal sample and buffer to a medical diagnostics laboratory.

85-86. (canceled)

87. (currently amended) A method of processing a fecal sample, the method comprising:

A) obtaining a pair of portions of a fecal sample from a human subject, the pair of portions comprising:

i) a sealed sealable container containing a stabilized removed portion and a buffer, and

ii) a sealed sealable collection vessel containing a stabilized remaining portion of a fecal sample and a buffer,

the pair of portions obtained by the method of claim 82,

B) testing the removed portion of said fecal sample for a concentration of hemoglobin present in said removed portion,

C) extracting DNA from the remaining portion of said fecal sample; and

D) analyzing said DNA for the presence of DNA from the human subject having an epigenetic modification.

88. (canceled)

89. (previously presented) The method of claim 87, wherein testing for the concentration of hemoglobin in said removed portion comprises immunochemical detection of hemoglobin.

90. (previously presented) The method of claim 87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 5 ng/ml.

91. (previously presented) The method of claim 87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 10 ng/ml.

92. (previously presented) The method of claim 87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 20 ng/ml.

93. (previously presented) The method of claim 87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 50 ng/ml.

94. (previously presented) The method of claim 87, wherein the removed portion of said fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in said removed portion is at least 200 ng/ml.

95. (canceled)

96. (previously presented) The method of claim 87, wherein said epigenetic modification comprises aberrant methylation.

97. (previously presented) The method of claim 87, wherein said aberrant methylation comprises hypermethylation.

98. (previously presented) The method of claim 87, wherein said DNA from the human subject comprises a gene and/or a promoter region of a gene.

99. (previously presented) The method of claim 98, wherein said gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.

100. (previously presented) The method of claim 96, wherein analyzing said DNA comprises modifying said DNA with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil.

REMARKS

In the Final Office Action mailed 03/25/2022 ("Office Action"), the Examiner made the following objections and rejections:

I. Claims 82, 84, 87, 96, and 97 are rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over US 2006/0210448 Al ("Wang") in view of US 2009/0004058 Al ("Liang"), US 2005/0064401 Al ("Olek") and Kutzner et al. ("Non-invasive detection of colorectal tumors by the combined application of molecular diagnostics and the faecal occult blood test", *Cancer Letters"* 229 (2005) 33-41; "Kutzner").

II. Claims 89-94 are rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97, and further in view of US 2006/0188939 Al ("Gao") and US 2008/0227208 Al ("Yee")[1].

III. Claims 98-100 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97, and further in view of either Kim (BMB reports, Volume 41, Issue 10, Pages 685-692, September 2008; hereinafter "Kim") or WO 2008/084219 Al ("Van Engeland").

Claim amendments

For business reasons and without acquiescing to the Examiner's arguments, and reserving the right to prosecute the original or similar claims in one or more future applications, claim 82 is herein amended to recite removing a portion of the fecal sample to a separate sealable container, and further to recite, after combining the removed portion with the recited buffer, sealing the

[1] In paragraphs 31 and 39 of the Office Action, the Examiner cited "Yeh (US 2008/0227298 Al)." Applicant believes the Examiner intended to cite US 2008/022720**8** A1 to Ye**e**, as the cited publication number names inventor Hirota and is directed to semiconductor device manufacturing. If the Examiner indeed meant to cite US 2008/0227298 A1 to Hirota, Applicant requests correction of the Office Action.

sealable container. Claim 82 is further amended to recite in part d), after adding the recited buffer, sealing the sealable collection vessel. Sealing of the sealable container containing the buffer-stabilized removed portion and the collection vessel containing the buffer-stabilized remaining portion is demonstrated, *e.g.*, in Example 1, in the paragraph bridging pages 43 and 44, as evidenced by the shipping of the separate buffer-stabilized portions of the collected fecal samples, the shipping necessitating sealing of the containers containing the samples.

Claims 84 and 87 are amended for consistency with claim 82.

These amendments do not add new matter.

Applicant respectfully requests entry of these amendments in accordance with 37 C.F.R. 1.116 (b)(2).

The claims are not obvious

I. Claims 82, 84, 87, 96, and 97 are rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner. Applicant respectfully disagrees that the cited references render the claims *prima facie* obvious.

It is well-settled law that establishing *prima facie* obviousness requires *at least* a suggestion of all of the features in a claim. *See In re Wada and Murphy*, *citing CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) and *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). See also MPEP §2143. Further, a valid *prima facie* case requires the Office to present "articulated reasoning with some rational underpinning", *KSR*, 550 U.S. at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)), that one of skill in the art would have been motivated to combine the elements to yield the claimed combination and that one would have done so having a reasonable expectation of successfully carrying out the claimed combination. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

As discussed above, the instant claims as amended are directed to a method of processing a freshly-collected fecal sample from a subject without freezing, wherein the sample is collected at home by the subject by defecation directly into a sealable collection vessel, wherein the sample is divided into portions that are separately stabilized, *e.g.*, for preserving different types

of analytes in each portion. In particular, a removed portion in a separate container is combined with a buffer that stabilizes blood proteins and the remaining portion is separately treated in the original collection vessel with a buffer that maintains DNA integrity in the fecal sample. A fecal sample collected in the claimed manner is suitable for detecting blood proteins in one portion and DNA in the other portion of the same fecal sample.

Applicant's claim 82 recites use of two different kinds of sealable containers:

1) a sealable collection vessel, and
2) a sealable container

As set forth in claim 82, the method requires that a fecal sample collected be at home by a human subject by defecation directly into the sealable collection vessel. The claim further recites that, after removal of the "removed portion" of the fecal sample, the "remaining portion" of the fecal sample is stabilized by addition of a buffer to the sealable collection vessel.

Claim 87 recites a pair of portions obtained by the method of claim 82, the pair of portions comprising:

i) a sealed sealable container containing a stabilized removed portion and a buffer, and
ii) a sealed sealable collection vessel containing a stabilized remaining portion of a fecal sample and a buffer.

The Examiner points to Wang as teaching a device for collecting a portion of a fecal sample and use of a preserving fluid, but admits that Wang does not teach collecting pairs of samples or sending pairs of samples to a lab for testing (Office Action, ¶17).

Applicant respectfully points out that Wang also fails to teach use of a sealable collection vessel as set forth in instant claims 82 and 87, collection of a sample by direct defecation into the sealable collection vessel, stabilization of a "remaining portion" of a fecal sample by buffer in the sealable collection vessel, or delivery of the sealable collection vessel containing the buffer-stabilized sample together with the buffer-stabilized removed portion to a lab for testing, as recited in claim 84.

Instead, the only sample collection device contemplated by Wang is suited only for

removing a small portion of a fecal sample using "stick 18", as illustrated in Figs. 1 and 2 of Wang, and having a "distal portion 21" having a groove to hold a small portion of a fecal sample (see, e.g., Wang at ¶25).

To the extent that Wang teaches catching a fecal sample using a toilet, Wang teaches that the catching is only for the purpose of temporarily catching the sample so that a portion can be removed by the disclosed stick device. For this purpose, Wang teaches providing a disposable paper catch web "temporarily secured to a toilet droopingly spanning the seat to catch the feces for sampling" (Office Action ¶15, referencing the Abstract of Wang). Wang specifies that the disposable web 61 is preferably made of a flushable paper (Wang, ¶34. See also Wang Fig. 7). Thus, the disposable web 61 of Wang is not a "sealable collection vessel" that can hold a buffer. Further, Wang does not suggest either preserving the remainder of the feces after collection of a portion using the disclosed sampling device, nor sending the disposable web 61 with the feces to a laboratory. Rather, Wang clearly points to disposing of the remaining feces, preferably by flushing it away with disposable web 61.

Further, the deficiencies of Wang with respect to using a combination of a sealable collection vessel and a sealable container as set forth in instant claims 82 and 87 is not remedied by combination of Wang with any of the other cited references.

The Examiner points to Liang for a teaching of a device and method for collecting and testing fecal samples (Office Action, ¶18). However, even if Liang discloses collection of multiple samples using several of the collection devices, as asserted by the Examiner, Liang suffers from the same deficiencies as Wang. Liang discloses only collecting small portions of feces using a "sampling wand" of the disclosed device. (Liang, ¶112).

Liang teaches that the user collects his/her feces on a "flushable paper sheet secured across the seat of a toilet," and uses a sampling wand inserted into the feces to collect the small portions for testing. The flushable paper sheet of Liang is not a "sealable collection vessel" that can hold a buffer. Further, Liang does not suggest either preserving the remainder of the feces after collection of a portion using the disclosed sampling wand, nor sending the flushable paper sheet with the feces to a laboratory. Rather, Liang clearly points to disposing of the remaining feces, preferably by flushing it away with flushable paper sheet.

Liang does not teach or suggest use of a sealable collection vessel as set forth in instant

claims 82 and 87, collection of a sample by direct defecation into the sealable collection vessel, stabilization of a "remaining portion" of a fecal sample by buffer in the sealable collection vessel, or delivery of the sealable collection vessel containing the buffer-stabilized sample together with the buffer-stabilized removed portion to a lab for testing as recited in claim 84. Thus, no matter how many of the devices of Wang and/or Liang are used to collect samples from feces, the combination of Wang and Liang still fails to teach of suggest all the elements of claims 82, 84 and 87 and the claims depending therefrom.

The Examiner points to Kutzner as disclosing detection of colorectal tumors using a combination of tests, one which looks for a component of blood, and one which is based on nucleic acid analysis, e.g., PCR analysis. (Office Action, ¶14).

While not acquiescing to the Examiner's characterization of Kutzner, Applicant points out that Kutzner describes a retrospective study of mutations and blood in frozen patient samples (Kutzner, p34, col 2). Kutzner does not teach or suggest a method of processing a freshly-collected fecal sample without freezing using a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate sealable container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, as recited in instant claim 82, delivery of the containers to a laboratory as recited in claim 84, nor processing of such samples as set forth in claim 87.

Further, as acknowledged by the Examiner, Kutzner also does not suggest testing a fecal sample for a concentration of hemoglobin, (Office Action, ¶33), much less testing a removed portion collected as described in the instant claims for a concentration of hemoglobin. Kutzner also does not teach assaying DNA for aberrant methylation.

Kutzner clearly states that their "retrospective data *do not allow any conclusion* on the potential of this approach [combined application of molecular diagnosis (MD) and fecal occult blood test (FOBT)] in diagnostic screening" and state that "Prospective comparative studies, which include a very high number of individuals, have to prove the value of the combined application of MD and FOBT." (Kutzner, p40, paragraph bridging columns 1-2, emphasis added). Thus, Kutzner specifically states that it is not obvious to the authors of the reference, as

skilled artisans, that combined testing of MD markers with FOBT will yield any particular result with respect to diagnostic screening. Kutzner provides no more than a guess that the approach *might be* successful, but does not provide a "reasonable expectation of success" required to establish *prima facie* obviousness.

The Examiner points to Olek as teaching performing tests on stool samples whereby one detects DNA sequence. (Office Action, ¶22-25). However, Olek does not teach anything about either testing for hemoglobin, nor methods of collecting or processing fecal samples as set forth in Applicant's claims 82 and 87 and the claims depending therefrom. Olek therefore cannot cure the deficiencies of Wang in combination with Liang and Kutzner with respect to the missing elements.

While not acquiescing that the other requirements for establishing *prima facie* obviousness have been met, Applicant submits that, for the reasons set forth above, the combination of Wang in view of Liang, Olek, and Kutzner does not set forth the elements of Claims 82, 84, 87, 96, and 97 and, for at least this reason, does not render these claims *prima facie* obvious. Applicant therefore respectfully requests that the rejection be withdrawn.

II. Claims 89-94 are rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97, and further in view of Gao and Yee.

Applicant disagrees for at least the reason that Gao and Yee do not cure the deficiencies of Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97.

The Examiner points to Gao as teaching methods of detecting analytes, including hemoglobin, in a fecal sample. The Examiner points to Yee as teaching setting a 10 ng/ml of hemoglobin in blood as a threshold value. (Office Action, ¶¶ 34-36).

However, neither Gao nor Yee teach or suggest a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate sealable container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, as recited in

instant claims 82, or processing of such samples as set forth in claim 87. Claims 89-94 depend from claim 87 and thereby comprise the same elements.

Thus, while not acquiescing that the other requirements for establishing *prima facie* obviousness have been met, for the reasons set forth above Applicant submits that the combination of Wang in view of Liang, Olek, and Kutzner as applied to Claims 82, 84, 87, 96, and 97 and further in view of Gao and Yee fails to teach all the elements of claims 89-94 and thus does not render these claims *prima facie* obvious. Applicant therefore respectfully requests that the rejection be withdrawn.

III. Claims 98-100 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97, and further in view of either Kim or Van Engeland.

Applicant disagrees for at least the reason that Kim and Van Engeland do not cure the deficiencies of Wang in view of Liang, Olek, and Kutzner, as applied to claims 82, 84, 87, 96, and 97.

The Examiner errs in citing Van Engeland. (Office Action, ¶¶ 40, 44, 52). Applicant has previously established that Van Engeland, being filed less than one year prior to the priority date of the instant application could qualify as prior art *only* under pre-AIA 35 U.S.C. § 102(a). Applicant has further established by Affidavit that the Van Engeland does not disclose invention "by another," as required under pre-AIA 35 U.S.C. § 102(a), and therefore does not qualify as prior art to the present application. Applicant directs the Examiner to Applicant's Response filed 11/29/2021 and to the Inventor's Declaration Under 37 C.P.R. § 1.132 by inventor of the present invention Joost Louwagie ("Louwagie Decl."), which was originally filed with the Office in Appl. Ser. No., 13/147,570, to which the instant application claims priority as a continuation of U.S. Application No. 15/010,436, filed January 29, 2016, which is a continuation of U.S. Application No. 13/147,570.

Furthermore, even if Van Engeland is considered in the present rejection as disclosing the marker NDRG4, Van Engeland fails to cure the deficiencies of Wang in view of Liang, Olek, and Kutzner, discussed in detail above, with respect to the missing elements of the instant claims.

The Examiner points to Kim as allegedly teaching colorectal cancer epigenetic markers. (Office Action, ¶43). However, Kim does not cure the deficiencies of Wang in view of Liang, Olek, and Kutzner.

Examiner points to Kim as allegedly teaching "colorectal cancer epigenetic biomarkers are used for the early detection of colorectal cancer with fecal-occult blood test (FOB)" pointing to page 686, Table 1, particularly column 1 bridging column 2 of page 689. (Office Action, ¶43). Applicant respectfully disagrees with the Examiner's characterization of Kim, as Kim makes no mention of testing epigenetic biomarkers along with FOB *on the same fecal specimen*. Rather, Kim is a review article that catalogues the "noninvasive molecular biomarkers that are currently in clinical settings and have the potential for use as CRC biomarkers" as of the time Kim was published in 2008 (Kim, Abstract). While Kim references the NDRG4 marker, nothing in Kim teaches or suggests *combining* detection of NDRG4 (or any other colorectal cancer epigenetic biomarkers) with fecal-occult blood testing on separately stabilized portions of the same freshly collected fecal sample, as recited in Applicant's claims.

Further, Kim does not teach or suggest a method comprising collection of a fecal sample at home by a human subject by defecation directly into a sealable collection vessel, or wherein a portion of the fecal sample is removed to a separate sealable container and combined with a buffer that stabilizes blood proteins, while the portion remaining in the sealable collection vessel is combined with a buffer that maintains DNA integrity in the fecal sample, as recited in instant claims 82, or processing of such samples as set forth in claim 87. Claims 98-100 depend from claim 87 and thereby comprise the same elements.

Thus, while not acquiescing that the other requirements for establishing *prima facie* obviousness have been met, for the reasons set forth above Applicant submits that the combination of Wang in view of Liang, Olek, and Kutzner as applied to Claims 82, 84, 87, 96, and 97 and further in view of Kim fails to teach all the elements of claims 98-100 and thus does not render these claims *prima facie* obvious. Applicant therefore respectfully requests that the rejection be withdrawn.

Applicant's alleged admissions

In addition to the references discussed above, the Examiner characterized a number of

items from Applicant's Specification as applicant admissions. See, e.g., Office Action at ¶¶ 45-48. 46. While the alleged applicant admissions are not enumerated or discussed here, the absence of discussion in this response is not to be taken as a concession that any information from Applicant's Specification is conceded by the Applicant to be prior art to the instant claims, nor that the Examiner's characterization of any such alleged admission is correct.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that all grounds for rejection have been addressed and Applicant's claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned at (608) 662-1277.

Dated: May 25, 2022 /Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
608.662.1277

Electronic Acknowledgement Receipt

EFS ID:	45802214
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow
Filer Authorized By:	
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	25-MAY-2022
Filing Date:	27-JUN-2017
Time Stamp:	14:49:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	After Final Consideration Program Request	2022-05-22_EXCTD-35239-304_AFCP_Form.pdf	226487 bb076be1285e9653f060373a541587726ca79346	no	2

Warnings:

Information:					
2		2022-05-25_RFOA_EXCTD-003 USCON2-35239_35239-304.pdf	174010 84f0ba537ce004cc19bb6085b87c4befc4b6 91b2	yes	13

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	13

Warnings:	
Information:	
Total Files Size (in bytes):	400497

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/634,607	Filing Date 06/27/2017	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	05/25/2022	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 14	Minus	** 20	= 0		x $0 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $0 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/YOLANDA CHADWICK/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 06/14/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
SISSON, BRADLEY L	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
06/14/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@CASIMIRJONES.COM
Genomic_docketing@cardinal-ip.com

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 15/634,607	**Applicant(s)** Louwagie, Joost	
	Examiner Bradley L Sisson	**Art Unit** 1634	**AIA (FITF) Status** No

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 25 May 2022 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☑ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:
   a) ☑ The period for reply expires 3 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection.The current period for reply expires ____ months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.
   Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANTS FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37CFR 41.37(a).

AMENDMENTS

3. ☑ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   a) ☑ They raise new issues that would require further consideration and/or search (see NOTE below);
   b) ☐ They raise the issue of new matter (see NOTE below);
   c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: See Continuation Sheet (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☑ For purposes of appeal, the proposed amendment(s):(a) ☑ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____
9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☑ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
14. ☑ Other: See Continuation Sheet.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:
    Claim(s) allowed:_____.
    Claim(s) objected to:_____.
    Claim(s) rejected:82,84,87,89-94 and 96-100.
    Claim(s) withdrawn from consideration:_____.

/Bradley L. Sisson/ Primary Examiner, Art Unit 1634	

Continuation of 3. NOTE: The aspect of adding new limitation to independent claim 82 whereby the container is sealable would require further consideration and/or search

Continuation of REQUEST FOR RECONSIDERATION/OTHER 12. The request for reconsideration has been considered but does NOT place the application in condition for allowance because: Applicant's traversal of the rejection has been found to be predicated on the entry of the amendment. As indicated above, the amendment has not been entered. Therefore, the rejections are maintained for reasons of record.

Continuation of REQUEST FOR RECONSIDERATION/OTHER 14. Other: Attached:
a) PTO-892 Notice of references Cited
b) PTO-413 Interview Summary
c) PTO-2323 After-Final Consideration Pilot

Examiner-Initiated Interview Summary	**Application No.** 15/634,607	**Applicant(s)** Louwagie, Joost		
	Examiner Bradley L Sisson	**Art Unit** 1634	**AIA (First Inventor to File) Status** No	**Page** 1 of 2

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
Bradley L Sisson	Primary Examiner	Telephonic
Mary Ann D. Brow, Reg. No. 42,363	Attorney of Record	

Date of Interview: 09 June 2022

Issues Discussed:

Proposed Amendment(s)

The amendment of 25 May 2022, filed under 37 CFR 1.116, was discussed. Ms. Brow directed attention to the limitation that a human defecates "directly into a sealable collection vessel", and that a "portion" of the fecal sample is removed to a "separate sealable container".

Examiner Sisson indicated that in conducting an updated search of prior art that several relevant documents had been identified. Those documents are:

a) US 2004/0019298 A1 (Zhou et al.), in the abstract and at paragraph [0025], teach collection of a fecal sample and that the collection device is loadable into a sealable shipping capsule.

b) US 6,761,702 B2 (Smith), in th abstract and at column 2, fifth paragraph, teaches of colonic lavage and that the fluid and cleansed fecal material is passed to a sealable container.

c) US 2005/0075543 A1 (Calabrese), at paragraph [0040], teaches of a kit for collection of sample at home, the use of a sealable container, and that "The urine or stool sample is collected y collecting enough of he specimen in the sample container." And that in paragraph [0052], it is disclosed that " Alternatively, the patient may use a home kit to obtain and submit the sample..."

d) US 2003/0086869 A1 (Stallings et al.), in paragraph [0086], teach of 72-hour stool collection and that it is collected and initially stored at home.

e) US 4,445,235 (Slover et al.) teaches of a collection device that inserts into a toilet.

f) US 2007/0017015 A1 (Finell), in paragraph [0082], teaches of a collection chamber in a potty, and that one defecates directly into the the chamber.

Examiner Sisson noted that the instant application had not been filed with any drawings/figures, much less figures that describe the "sealable collection vessel" that one is to defecate "directly into".

Ms. Brow directed attention to page 23, first paragraph, line 7. of the as-filed disclosure which refers to " a special bucket".

Examiner Sisson noted that while the specification may refer to "a special bucket", such does not describe how it is designed not only for direct defecation, but for sealing upon defecation and resealing of the "sealable collection vessel" (proposed claim 82) once a sample had been removed therefrom and placed into a separate sealable container.

While not discussed during he interview, attention is also directed to US 2008/0097238 A1 (Loktionov et al.), at paragraph [0013], teaches of isolating exfoliated cells from the surface of human stool. As stated therein:

"A method of exfoliated cell isolation from human whole stool samples by washing cells off the surface of cooled faeces and collecting them by immunomagnetic separation procedure has been developed [Loktionov, A. et al., Clin. Cancer Res. 1998; 4: 337-342]. Although work in this direction was initially planned in terms of developing a molecular diagnostic assay for CRC, it emerged that a simple quantitative analysis of colonocyte-derived DNA from human stool surface could be used for CRC diagnosis and screening since the relative DNA amount in CRC patients was much higher compared to healthy individuals."

Attention is also directed to US 2003/0096244 A1 (Rabello et al.), which teaches at paragraph [0037], of isolating from human feces cells from a parasite, and ultimately the DNA from within the parasitic cells, wherein the parasite is Schistosoma.

Such teachings arguably provide motivation for one to not only obtain a small, surface sample of fecal material for human DNA for signs of disease (e.g., colon cancer), as well as obtain and test the large sample for cells, and the DNA contained therein, for the detection of parasites in the larger sample portion.

Other

Ms. Brow indicated that applicant may file an appeal or file a pre-appeal brief review request and conference.

/Bradley L. Sisson/ Primary Examiner, Art Unit 1634	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Notice of References Cited	Application/Control No. 15/634,607	Applicant(s)/Patent Under Reexamination Louwagie, Joost	
	Examiner Bradley L Sisson	Art Unit 1634	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20040019298-A1	01-2004	Zhou, David F.	A61B10/0096	600/564
*	B	US-6761702-B2	07-2004	Smith; Alan	A61M1/774	604/35
*	C	US-20050075543-A1	04-2005	Calabrese, Charles A.	G16H10/40	600/300
*	D	US-20030086869-A1	05-2003	Stallings, Virginia A.	C12Q1/44	424/9.1
*	E	US-4445235-A	05-1984	Slover; Pearl	A61B10/0038	4/144.1
*	F	US-20070017015-A1	01-2007	Finell; Rebecca M.	A47K11/06	4/483
*	G	US-20080097238-A1	04-2008	Loktionov; Alexandre	A61B10/0096	600/562
*	H	US-20030096244-A1	05-2003	Rabello, Ana Lucia Teles	C12Q1/6888	435/7.22
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

AFCP 2.0 Decision	Application No.	Applicant(s)	
	15/634,607	Louwagie, Joost	
	Examiner	Art Unit	AIA (FITF) Status
	Bradley L Sisson	1634	No

This is in response to the After Final Consideration Pilot request filed 25 May 2022.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ The request is not the first proper AFCP 2.0 request submitted in response to the most recent final rejection.

   ☐ Other: _____

2. **Proper Request**

   A. After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☐ The after final amendment will be treated under pre-pilot procedure.

   B. Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☐ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action . See attached interview summary for further details.

   ☑ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other: _____

   Examiner Note: Please attach an interview summary when necessary as described above.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.
EAST	06/16/2021	B. L. S.
EAST	03/22/2022	B. L. S.
PE2E Search	06/09/2022	B. L. S.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Bradley L Sisson	1634

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022		
	1	-	-	-	-	-	-	-		
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	41	-	-	-	-	-	-	-		
	42	-	-	-	-	-	-	-		

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022		
	43	-	-	-	-	-	-	-		
	44	-	-	-	-	-	-	-		
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	79	-	-	-	-	-	-	-		
	80	-	-	-	-	-	-	-		
	81	-	-	-	-	-	-	-		
	82	÷	✓	✓	✓	✓	✓	✓		
	83	÷	✓	✓	✓	✓	-	-		
	84	÷	✓	✓	✓	✓	✓	✓		
	85	÷	-	-	-	-	-	-		
	86	÷	-	-	-	-	-	-		
	87	÷	✓	✓	✓	✓	✓	✓		
	88	÷	✓	✓	✓	✓	-	-		
	89	÷	✓	✓	✓	✓	✓	✓		
	90	÷	✓	✓	✓	✓	✓	✓		
	91	÷	✓	✓	✓	✓	✓	✓		
	92	÷	✓	✓	✓	✓	✓	✓		
	93	÷	✓	✓	✓	✓	✓	✓		
	94	÷	✓	✓	✓	✓	✓	✓		

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
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	Examiner	**Art Unit**
	Bradley L Sisson	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022		
	95	÷	✓	✓	✓	✓	-	✓		
	96	÷	✓	✓	✓	✓	✓	✓		
	97	÷	✓	✓	✓	✓	✓	✓		
	98	÷	✓	✓	✓	✓	✓	✓		
	99	÷	✓	✓	✓	✓	✓	✓		
	100	÷	✓	✓	✓	✓	✓	✓		

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	53	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 10:50 AM
L2	1	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same "ng/ml" and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 10:51 AM
L3	9	(hemoglobin haemoglobin) with concentration same ((stool fecal faecal) adj sample) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 11:18 AM
L4	260	DNA same ((stool fecal faecal) adj sample) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 11:35 AM
L5	2	"20080227208"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 01:34 PM
L6	1	"20080227208" and "10 ng/ml"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 01:35 PM
L7	11	(hemoglobin haemoglobin) with "5 ng/ml"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2019/06/23 01:38 PM
L8	0	NDGR4 and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 01:50 PM
L9	38	NDRG4 and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 01:50 PM
L10	6	NDRG4 same epigenetic and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 01:58 PM
L11	24	"2006113770"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:02 PM

L12	4	"2006113770" and NDRG4	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:11 PM
L13	6	NDRG4 same (epigenetic methylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:19 PM
L14	6	NDRG4 same (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:19 PM
L15	0	"N-myc downstream regulated gene 4" same (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:24 PM
L16	0	"N-myc downstream regulated gene 4" and (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:24 PM
L17	0	"N-myc downstream regulated gene 4" and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:25 PM
L18	1	"20060019256" and uptg	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 02:55 PM
L19	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/14 03:35 PM
L20	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/15 08:07 AM
L21	3	fecal same (antibody antibodies immunoassay) same (DNA RNA polynucleotide "nucleic acid" gene) same (hyridization hybridizes hybridize) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/15 08:26 AM

L22	2	(cancer carcinoma) same (PHACTR3 NDRG4 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFRP2 CDO1) same (hyridization hybridizes hybridize) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/15 10:40 AM
L23	1	"200483399"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/15 10:42 AM
L24	0	"200483399A2"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/01/15 10:43 AM
L25	0	"200483399A2"	(EPO)	OR	OFF	OFF	2020/01/15 11:11 AM
L26	0	"200483399"	(EPO)	OR	OFF	OFF	2020/01/15 11:11 AM
L27	40	(PHACTR3 FOXE1 GATA4 GPNMB TFP12 SOX17 SYNE1 LAMA MMP2 OSMR SFR2CDO1) same (epigenetic methylation hypermethylation) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/08/04 06:14 PM
L28	38	NDRG4 and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2020/08/04 07:10 PM
L29	15	hemoglobin with (stool fecal) with concentration and @pd<"20090203"	(USPAT)	OR	OFF	OFF	2021/06/15 10:13 AM
L30	32	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) and (antibody antibodies immunoassay immunological) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 11:33 AM
L31	16	(hemoglobin haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 11:33 AM
L32	1	(hemoglobin	(US-PGPUB; USPAT;	OR	ON	ON	2021/06/16

		haemoglobin) with concentration same (stool fecal faecal) same (antibody antibodies immunoassay immunological) and "ng/ml" and @pd<"20090203"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:37 AM
L33	1048	(freeze freezing frozen) same (stool fecal faecal) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 12:05 PM
L34	399	(freeze freezing frozen) with (stool fecal faecal) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 12:05 PM
L35	236	(freeze freezing frozen) with (stool fecal faecal) with (sample fraction portion historical) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 12:07 PM
L36	14	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion historical) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 12:07 PM
L37	4	(immunoassay elisa) with (stool fecal faecal) with (fresh "non-frozen") and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 12:31 PM
L38	1	"7832219".FMID.	(US-PGPUB; USPAT; FPRS; JPO)	OR	OFF	OFF	2021/06/16 12:32 PM
L39	4	"7832219"	(USPAT)	OR	ON	ON	2021/06/16 12:33 PM
L40	2	(freeze freezing frozen) with (stool fecal faecal) with (fraction portion historical) same buffer and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 01:30 PM
L41	5	(freeze freezing frozen) same (stool fecal faecal) with (fraction portion historical) same buffer and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 01:33 PM
L42	230	"5741650"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/16 01:38 PM
L43	114	"5741650"	(USPAT)	OR	ON	ON	2021/06/16 01:38 PM
L44	63	"5741650" and frozen	(USPAT)	OR	ON	ON	2021/06/16 01:40 PM

L45	65	"5741650" and (freeze freezing frozen)	(USPAT)	OR	ON	ON	2021/06/16 01:41 PM
L46	3	(feces fecal faecal faeces) with buffer with (freeze freezing frozen) and @pd<"20090203"	(USPAT)	OR	ON	ON	2021/06/16 01:51 PM
L47	0	fecal with sample same (unfrozen "not frozen") and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/17 02:40 PM
L49	85	fecal with sample same (frozen freezing) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/17 02:42 PM
L50	12	fecal with sample same (sealable sealed seal) with (vessel container) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/17 02:57 PM
L51	17	fecal with sample same (unfrozen "not frozen" freezing) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 03:56 PM
L52	4	"20080124714"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 03:58 PM
L53	6	fecal with sample same (sealable sealed seal) with (vessel container) same portion and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 04:18 PM
L54	179	(stool fecal faecal) with sample with portion and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 04:52 PM
L55	2	(stool fecal faecal) with sample with portion with (multiple duplicate) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/21 04:54 PM
L56	69546	((dna with (isolate extract extracted isolating)) (determine determining determins determined) with (hemoglobin haemoglobin) same (fecal faecal)) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 05:27 PM
L57	1	((dna with (isolate extract extracted isolating)) and (determine determining determins determined) with (hemoglobin	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/21 05:28 PM

		haemoglobin) same (fecal faecal)) and @pd<"20090203"					
L58	128	DNA same "epigenetic modification" and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/22 05:47 PM
L59	1	DNA same "epigenetic modification" and (faecal fecal) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/22 05:47 PM
L60	20	DNA same "epigenetic modification" and (faecal fecal stool) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/22 05:48 PM
L61	21	DNA same "epigenetic modification" and (faecal fecal stool feces) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/22 05:48 PM
L62	19	DNA same "epigenetic modification" and (dna "nucleic acid" chromosomal chromatin) same (faecal fecal stool feces) and @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2022/03/22 05:49 PM
L63	0	"20050064401" and (hemoglobin haemoglobin)	(US-PGPUB)	OR	OFF	OFF	2022/03/22 06:07 PM
L64	112	immunoassay same (hemoglobin haemoglobin) and @pd<"20090203"	(US-PGPUB)	OR	OFF	OFF	2022/03/22 06:33 PM
L65	51	immunoassay with (hemoglobin haemoglobin) and @pd<"20090203"	(US-PGPUB)	OR	OFF	OFF	2022/03/22 06:33 PM
L66	2	immunoassay with (hemoglobin haemoglobin) same (stool fecal faecal) and @pd<"20090203"	(US-PGPUB)	OR	OFF	OFF	2022/03/22 06:44 PM
L67	87	sealable WITH (container vessel) AND fecal AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/06 04:19 PM
L68	14	sealable WITH (container vessel) SAME fecal AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/06 04:19 PM
L69	0	sealable WITH (container vessel) SAME (deficate	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 07:15 AM

		defication) AND @pd<"20090203"					
L70	0	sealable AND (container vessel) SAME (deficate defication) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 07:16 AM
L71	0	(container vessel) SAME (deficate defication) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 07:16 AM
L72	0	(container vessel) SAME (deficate defication) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 07:16 AM
L73	12306	(container vessel) SAME (stool fecal) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 07:40 AM
L74	1992	(container vessel) SAME (stool fecal) SAME (full complete entire) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA,	OR	ON	ON	2022/06/07 07:41 AM

			VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L75	439	(container vessel) SAME (stool fecal) WITH (full complete entire) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 07:42 AM
L76	76	(container vessel) SAME (stool fecal) WITH (full complete entire) AND (container vessel) WITH (seal sealable) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 07:42 AM
L77	77	(container vessel) SAME (stool fecal "night soil") WITH (full complete entire) AND (container vessel) WITH (seal sealable) AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 10:15 AM
L78	1801	(container vessel) SAME (stool fecal "night soil") AND home AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2022/06/07 10:36 AM

			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L79	13	(container vessel) SAME (stool fecal "night soil") SAME sealable AND home AND @pd<"20090203"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/06/07 10:36 AM
L80	0	(deficate deficat$) SAME container AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:17 AM
L81	3	(deficate deficat$) SAME collection AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:18 AM
L82	1884	(deficate deficat$ "night soil" stool) SAME (deposit$ collect$) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:20 AM
L83	363	(deficate deficat$ "night soil" stool) SAME (deposit$ collect$) WITH (chamber device tray container) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:21 AM
L84	26	(deficate deficat$ "night soil" stool) SAME (deposit$ collect$) WITH (chamber device tray container) SAME home AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:21 AM
L85	171	(deficate deficat$ "night soil" stool feces faeces) SAME (directly direct) WITH (chamber device tray containe vessel) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 11:53 AM

L86	2	"20070017015" AND chamber	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 12:02 PM
L87	2	"20070017015" AND chamber SAME (detach$ seal$)	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 12:04 PM
L88	0	"20070017015" AND chamber SAME (detach$ seal$) AND empt$	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 12:05 PM
L89	0	"20070017015" AND chamber SAME (detach$ seal$) AND empty	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 12:08 PM
L90	0	"20070017015" AND chamber SAME (detach$ seal$) AND empt$	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/07 12:08 PM
L91	171	(deficate deficat$ "night soil" stool feces faeces) SAME (directly direct) WITH (chamber device tray containe vessel) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 01:53 PM
L92	0	(deficate deficat$) WITH container AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 01:54 PM
L93	0	(deficate deficat$) WITH (vessel container) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 01:54 PM
L94	0	(deficate deficat$) SAME (vessel container) AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 01:54 PM
L95	26	fecal SAME (DNA RNA) WITH surface AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:12 PM
L96	1	fecal SAME (DNA RNA) WITH surface SAME human AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:14 PM
L97	0	fecal SAME (DNA RNA) WITH helminth SAME human AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:21 PM
L98	1	fecal SAME (DNA RNA) WITH parasite SAME human AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:22 PM
L99	15	fecal SAME (DNA RNA) WITH within SAME human AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:23 PM
L100	1	fecal SAME (DNA RNA)	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09

		WITH parasite SAME human AND @pd<"20090203"					04:28 PM
L101	7	(feces fecal stool faecal) SAME (DNA RNA) WITH parasite SAME human AND @pd<"20090203"	(US-PGPUB; USPAT)	OR	ON	ON	2022/06/09 04:29 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

DO NOT ENTER: /B.L.S./
06/09/2022

PATENT
Attorney Docket No.: **EXCTD-35239.304**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

AMENDMENT UNDER 37 C.F.R. §1.116 AFTER FINAL OFFICE COMMUNICATION MAILED 03/25/2022 AND REQUEST FOR AFTER-FINAL CONSIDERATION UNDER AFCP 2.0

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This communication is an After Final Amendment under 37 C.F.R. §1.116(b)(2), responsive to the Final Office Communication mailed 03/25/2022, with a response due on or before 06/25/2022, and is filed with a Request for After-Final Consideration. Applicants respectfully request entry of this amendment.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: **EXCTD-35239.304**. This paragraph is a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Amendments to the Claims are reflected in the Listing of Claims beginning on Page 2;

Remarks begin on Page 5.

To: DOCKETING@CASIMIRJONES.COM,Genomic_docketing@cardinal-ip.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 173254

Jun 14, 2022 12:33:55 PM

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UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
15634607	CTAV	06/14/2022	EXCTD-35239.304
	EXIN	06/14/2022	EXCTD-35239.304
	892	06/14/2022	EXCTD-35239.304
	A.NE.AFCP.D	06/14/2022	EXCTD-35239.304

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NOTICE OF APPEAL FROM THE EXAMINER TO THE PATENT TRIAL AND APPEAL BOARD

Docket Number (Optional): EXCTD-35239.304

I hereby certify that this correspondence is being facsimile transmitted to the USPTO, transmitted via the USPTO's patent electronic filing system, or deposited with the United States Postal Service with sufficient postage in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on 06/27/2020.

Signature: /Mary Ann D. Brow/

Typed or printed name: Mary Ann D. Brow

First Named Inventor	
LOUWAGIE	
Application Number: 15/634,607	Filed: 27-JUNE-2017
For: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD	
Art Unit: 1634	Examiner: SISSON

Applicant hereby **appeals** to the Patent Trial and Appeal Board from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 840 (previously paid)

☐ Applicant asserts small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by 50%, and the resulting fee is: $ _____

☐ Applicant certifies micro entity status. See 37 CFR 1.29. Therefore, the fee shown above is reduced by 75%, and the resulting fee is: $ _____
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 504302.

☑ Payment made via USPTO's patent electronic filing system (Patent Center or EFS-Web).

☐ A petition for an extension of time under 37 CFR 1.136(a) (PTO/AIA/22 or equivalent) is enclosed.
For extensions of time in reexamination proceedings, see 37 CFR 1.550.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant

☑ attorney or agent of record
Registration number 42363

☐ attorney or agent acting under 37 CFR 1.34
Registration number _____

Signature: /Mary Ann D. Brow/

Typed or printed name: Mary Ann D. Brow

Telephone Number: 608-662-1277

Date: 06/27/2022

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

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Electronic Acknowledgement Receipt

EFS ID:	46057078
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow
Filer Authorized By:	
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	27-JUN-2022
Filing Date:	27-JUN-2017
Time Stamp:	19:26:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Pre-Appeal Brief Conference Request	2022-06_27_PABRBrief_EXCTD-003USCON2-35239_35239-304.pdf	149097 50a4f4b0c7b5f9d58fe16178e78bf2b26dc87ed9	no	5

Warnings:

Information:					
2	Pre-Appeal Brief Conference Request	2022-06-27_35239-304_PABR_form.pdf	451829 9bf7896a66edf6311fcf8a90d81b4192f115e815	no	2
Warnings:					
Information:					
3	Notice of Appeal Filed	2022-06-27_35239-304_Notice Appeal.pdf	397891 f058ec6a2b931b1f785fb038967b8658066a3773	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	998817		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

APPLICANT INTERVIEW SUMMARY

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

A telephonic interview was conducted on June 9, 2022. Participants in the telephone call were Applicant's Representative Mary Ann D. Brow and Examiner Bradley Sisson. Claim 82 and after-final Amendment and Response filed on May 25, 2022 were discussed; no other exhibit was provided.

Applicant directed the Examiner's attention to the two different containers recited in claim 82, the sealable collection vessel in which a fecal sample is collected at home by the human subject by defecation directly into the sealable collection vessel, and the separate sealable container, used to contain a removed portion of the deposited fecal sample. Applicant further highlighted that the removed portion and the remaining portion are separately stabilized by combining with different types of buffers. Applicant pointed out that none of the cited references disclose a method comprising collection of a fecal sample at home by the human subject by defecation directly into a sealable collection vessel, or the recited method in which the freshly collected fecal specimen is portioned into a removed portion in a separate container and a remaining portion in the sealable collection vessel.

The Examiner pointed to several references not cited in the Final Office Action but communicated prior to the conference for discussion:

US 2004/0019298 A1 (Zhou et al.),

US 6,761,702 B2 (Smith),

US 2005/0075543 A1 (Calabrese),

US 2003/0086869 A 1 (Stallings et al.)

Applicant's representative noted that these references also do not disclose use a sealable collection vessel as set forth in the method of the claim, or the overall method of the claim.

The Examiner noted that the "sealable collection vessel" to receive a fecal sample by direct defecation was not described in detail. Applicant noted that patient defecation directly into a special bucket, followed by addition of a buffer and shipping to a laboratory is described on page 44, of the Experimental section of the Specification, and that the Examiner has not previously asserted any enablement or written description objection to this claim element.

No agreement regarding the patentability of the Claim 82 as amended was reached.

Dated: June 27, 2022 /Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
608.662.1277

Electronic Acknowledgement Receipt

EFS ID:	46057407
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow
Filer Authorized By:	
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	27-JUN-2022
Filing Date:	27-JUN-2017
Time Stamp:	20:06:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant summary of interview with examiner	2022-06-27_InterviewSummary_EXCTD-003USCON2-35239_35239-304.pdf	93889 cdfd22ff9fb3f03c9b6934bc9e22d51eeec65118	no	2
Warnings:					

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Office:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

PRE-APPEAL BRIEF REQUEST FOR REVIEW

Appellant requests a Pre-Appeal Brief Conference for the above-identified application. This is responsive to the Final Office Communication mailed 03/25/2022 ("Office Action") and the Advisory Action mailed 06/14/2022 ("Advisory Action"), with response due by Monday, 06/27/2022, the first business day following Saturday, 06/25/2022, and is submitted along with a Notice of Appeal in compliance with 37 CFR 41.31 and the Pre-Appeal Brief Conference form PTO/SB/33.

Claims 82, 84, 87, 89-94, and 96-100 are pending in the Application, with claims 82 and 87 being independent. Claims 82, 84, 87, 96, and 97 stand rejected under pre-AIA 35 U.S.C. 103(a) as allegedly being unpatentable over Wang in view of Liang, Olek, and Kutzner, with the remaining dependent claims being rejected under §103(a) over Wang, Liang, Olek, and Kutzner, in further view of one or more of Gao, Yee, Kim, and Van Engeland.

BASIS FOR REVIEW

The combination of references fails to teach or suggest all the elements of any claim. The Office points to Appellant's alleged admissions but these do not supply missing elements. The Office has provided no other evidence or argument sufficient to supply the missing elements of these claims and therefore fails to establish obviousness of any pending claim[1].

Appellant has recognized a need for collecting fresh fecal material from a subject at home, in a manner such that the material can be tested both for blood and for alterations in the subject's DNA. The claims are directed to methods of processing a fecal sample that is freshly collected at home by the subject, by defecation directly into a sealable collection vessel. A

[1] In the Advisory Action, the Office states that the arguments set forth in the after-final Response filed 05/25/2022 ("Response") are dependent on entry of amendments to the claims, which was denied. Appellant submits that the arguments are equally applicable to the unamended claims and does not waive these arguments.

portion of the collected sample is removed to a separate container, and the removed portion and the remaining portion are separately treated with appropriate buffers to stabilize different analytes in the feces, namely blood proteins and DNA.

Appellant's claim 82 recites, *inter alia,* two different containers and two kinds of buffer:

(1) a **sealable collection vessel** in which a fecal sample is collected at home by a human subject by defecation directly into the vessel;

(2) **a separate container**;

(3) **a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample**; and

(4) **a buffer that maintains DNA integrity in a fecal sample**

The claim also recites steps for producing *specific arrangements* of these elements with different portions the freshly-collected fecal sample. Namely, after the human subject has collected a fecal sample in the sealable collection vessel, a **removed portion** is taken from the collected fecal sample and combined with buffer (3) in the separate container (2)(as numbered above), and the **remaining portion** of the fecal sample in the sealable collection vessel (1) is combined with buffer (4), as described above. Claim 84 depends from claim 82 and therefore includes the same arrangements of elements, further reciting delivering the two different containers and the contained samples and buffers to a medical diagnostics laboratory.

Similarly, claim 87 recites a pair of portions of a fecal sample in two separate containers, specifically a container containing the removed portion and a buffer, and a sealable vessel containing the stabilized remaining portion, obtained by the method of claim 82. Thus, claim 87 also comprises the same elements and arrangement of elements set forth in claim 82, as discussed above. Claim 87 further recites steps of testing the removed portion for a concentration of hemoglobin, and testing DNA extracted from the remaining portion for DNA from the human subject that has epigenic modification. Claims 89-94 and 96-100 all depend from claim 87 and therefore comprise the same elements and arrangements thereof set forth in claims 82 and 87.

The primary combination of references cited in all rejections is Wang in view of Liang, Olek, and Kutzner. (Office Action ¶¶ 14, 31, and 40). The Office points to Wang as teaching a device for collecting a portion of a fecal sample and use of a preserving fluid, but admits that Wang does not teach collecting pairs of samples (Office Action, ¶17). However, the

shortcomings of Wang are far greater. Wang fails to teach or suggest a sealable collection vessel suitable to receive a fecal sample from human subject at home by defecation directly into the vessel; collection of a sample by direct defecation by the subject into the sealable collection vessel; removal of a "removed portion" from the fecal sample collected in the sealable collection vessel; or stabilization of a "remaining portion" of the fecal sample by adding buffer to the remaining portion in the sealable collection vessel. While Wang teaches a generic "preserving reagent" to "slow degradation of the biological molecule" (Wang at ¶3), Wang discloses neither a buffer "that prevents denaturation or degradation of blood proteins found in a fecal sample" nor "a buffer that maintains DNA integrity in a fecal sample," as recited in claim 82.

The sample collection device disclosed by Wang is suited only for collecting a portion of feces using "stick 18", as illustrated in Figs. 1 and 2 of Wang, stick 18 having a "distal portion 21" having a groove to hold a small portion of a fecal sample (see, *e.g.*, Wang at ¶25).

To the extent that Wang teaches catching defecated feces directly, Wang teaches only *temporarily* catching the feces so that a sample portion can be removed using the disclosed stick device. For this purpose, Wang teaches use of a disposable paper catch web "temporarily secured to a toilet droopingly spanning the seat to catch the feces for sampling" (Office Action ¶15, referencing the Abstract of Wang; See also Wang Figs. 7-8). Wang specifies that the disposable web 61 is preferably made of a flushable paper (Wang, ¶34). Thus, the disposable web 61 of Wang is not in any way a "sealable collection vessel" that can hold a buffer. Further, Wang suggests neither preserving the remainder of the caught feces after collection of a sample portion using the disclosed sampling device, nor any manner of sending the remaining feces to a laboratory. Rather, Wang clearly points to disposing of the remaining feces, preferably by flushing it away with disposable web 61.

Regarding Appellant's claim 84, the Office admits that Wang does not teach sending pairs of samples to a lab for testing (Office Action, ¶17). Appellant points out that Wang further fails to teach delivery of a pair of containers containing the pair of samples from the same freshly-collected fecal deposit, *i.e.,* sending the separate container containing the **removed portion** of the fecal sample combined with the buffer of (3) (as numbered above), and the sealable collection vessel containing the **remaining portion** of the fecal sample and the buffer of (4), to a medical diagnostics laboratory, as recited in this claim.

The deficiencies of Wang with respect to claims 82 and 87 and the claims depending

therefrom are not remedied by combination with Liang, Olek, and/or Kutzner. Liang suffers from the same deficiencies as Wang, disclosing only collecting a small portion of feces using a "sampling wand" (Liang, ¶112). Like Wang, Liang teaches that the user collects his/her feces on a "flushable paper sheet secured across the seat of a toilet," and uses the sampling wand inserted into the feces to collect a small portion for testing. The flushable paper sheet of Liang is not a "sealable collection vessel" that can hold a buffer. Further, Liang suggests neither preserving the remainder of the feces after collection of a portion using the sampling wand, nor sending the remaining feces to a laboratory. Rather, Liang clearly points to disposing of the remaining feces, preferably by flushing it away with flushable paper sheet.

The Office points to Liang ¶112 as allegedly disclosing that "one can collect multiple samples via a "sampling wand" (Office Action, ¶18). This mischaracterizes what Liang says. Liang says the sampling wand may be inserted into the fecal sample "multiple times in order to obtain sample from different portions of the [fecal] sample. The sampling wand (member)/cap portion is then reinserted into the test apparatus. . ." (Liang, ¶112; Office Action ¶18). Thus, in the procedure of Liang highlighted by the Office, fecal material collected on the sampling wand by the multiple insertions into the feces does not produce multiple *samples*, but rather produces a *single* sample comprising the combined material collected by the multiple insertions of the wand into the feces. This combined single sample is what is inserted into the test apparatus. Nothing in Liang suggests collecting multiple samples from the same fecal specimen for separate testing.

The Office asserts it would be obvious to combine the device of Wang with the device of Liang, to "capitalize on the fecal sample being accessible on the covered toilet and collection of multiple samples that can be sent independently to the testing facility for analysis." This is the entirety of the rationale provided. However, neither reference suggests portioning a single freshly-collected fecal sample into multiple separate samples or portions, nor suggests any benefit of sending such multiple samples independently to a testing facility for analysis. This statement is no more than a conclusory assertion of a motivation without any explanation of how it could arise from the cited documents. The Office's obviousness rationale thus appears to rely on hindsight reasoning based on Appellant's disclosure.

Furthermore, Liang does not teach or suggest use of a sealable collection vessel as set forth in instant claims 82 and 87, collection of a sample by direct defecation into the sealable collection vessel; stabilization of a "remaining portion" of a fecal sample by buffer in the

sealable collection vessel, or delivery of the sealable collection vessel containing the buffer-stabilized sample together with the buffer-stabilized removed portion to a lab for testing as recited in claim 84. Thus, no matter how many of the devices of Wang and/or Liang are used to collect samples from a fecal specimen, the combination of Wang and Liang still fails to teach or suggest all the elements of claims 82 and 87, and the claims depending therefrom.

The Office points to Olek as teaching performing tests on stool samples whereby one detects DNA sequence. (Office Action, ¶22-25), and to Kutzner as disclosing detection of colorectal tumors using a combination of tests, one which looks for a component of blood, and one which is based on nucleic acid analysis, *e.g.,* PCR analysis. (Office Action, ¶14). However, Olek does not teach anything about methods of collecting or processing fecal samples, and Kutzner describes a retrospective study of mutations and blood in frozen patient samples (Kutzner, p34, col 2). Further, Kutzner specifically states that it is not obvious to the authors of the reference that combined testing of DNA markers with fecal blood detection will yield any particular result with respect to diagnostic screening. Thus, neither Olek nor Kutzner cure the deficiencies of Wang, Liang, and Olek with respect to the missing elements discussed above.

The Office points to Gao as teaching methods of detecting analytes, including hemoglobin, in a fecal sample and to Yee as teaching setting a 10 ng/ml of hemoglobin in blood as a threshold value. (Office Action, ¶¶ 34-36), and to Kim or Van Engeland[2] and teaching epigenetic markers for colorectal cancer (Office Action, ¶¶43 and 44). As set forth in the Response, none of Gao, Yee, Kim, or Van Engeland cure the deficiencies of Wang in view of Liang, Olek, and Kutzner with respect to the missing elements discussed above.

In view of the points discussed above, it is clear that the Office has erred and has failed to establish *prima facie* obviousness of claims 82, 84, 87, 89-94, and 96-100.

Respectfully submitted,

Dated: June 27, 2022

/Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363
CASIMIR JONES, S.C.
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[2] Van Engeland does not disclose invention "by another," as required under pre-AIA 35 U.S.C. § 102(a), and therefore does not qualify as prior art to the present application.

Doc Code: AP.PRE.REQ

Document Description: Pre-Appeal Brief Conference Request

PTO/AIA/33 (03-22)
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PRE-APPEAL BRIEF REQUEST FOR REVIEW

Docket Number (Optional): EXCTD-35239.304

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on 06/27/2022

Signature /Mary Ann D. Brow/

Typed or printed name Mary Ann D. Brow

Application Number	Filed
15/634,607	27-June-2017

First Named Inventor: LOUWAGIE

Art Unit	Examiner
1634	SISSON

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

☐ applicant.

☑ attorney or agent of record. Registration number 42363.

☐ attorney or agent acting under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 _____

/Mary Ann D. Brow/
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Mary Ann D. Brow
Typed or printed name

608-662-1277
Telephone number

06/27/2022
Date

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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 08/10/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
SISSON, BRADLEY L	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
08/10/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

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Notice of Panel Decision from Pre-Appeal Brief Review

Application No.	Applicant(s)		
15/634,607	Louwagie, Joost		
Examiner	**Art Unit**	**AIA (FITF) Status**	
DAVE T NGUYEN	1634	No	

This is in response to the Pre-Appeal Brief Request for Review filed 27 June 2022 .

1. ☐ **Improper Request** - The Request is improper and a conference will not be held for the following reason(s):

☐ The Notice of Appeal has not been filed concurrent with the Pre-Appeal Brief Request.
☐ The request does not include reasons why a review is appropriate.
☐ A proposed amendment is included with the Pre-Appeal Brief request.
☐ Other: _____ .

The time period for filing a response continues to run from the receipt date of the Notice of Appeal or from the mail date of the last Office communication, if no Notice of Appeal has been received.

2. ☐ **Proceed to Board of Patent Appeals and Interferences** - A Pre-Appeal Brief conference has been held. The application remains under appeal because there is at least one actual issue for appeal. Applicant is required to submit an appeal brief in accordance with 37 CFR 41.37. The time period for filing an appeal brief will be reset to be one month from mailing this decision, or the balance of the two-month time period running from the receipt of the notice of appeal, whichever is greater. Further, the time period for filing of the appeal brief is extendible under 37 CFR 1.136 based upon the mail date of this decision or the receipt date of the notice of appeal, as applicable.

☐ The panel has determined the status of the claim(s) is as follows:
Claim(s) allowed: _____ .
Claim(s) objected to: _____ .
Claim(s) rejected: _____ .
Claim(s) withdrawn from consideration: _____ .

3. ☐ **Allowable application** - A conference has been held. The rejection is withdrawn and a Notice of Allowance will be mailed. Prosecution on the merits remains closed. No further action is required by applicant at this time.

4. ☑ **Reopen Prosecution** - A conference has been held. The rejection is withdrawn and a new Office action will be mailed. No further action is required by applicant at this time.

All participants:

(1) DAVE T NGUYEN .	(3) Bradley Sisson .
(2) Ashwin Mehta .	(4) _____ .

/DAVE T NGUYEN/ Supervisory Patent Examiner, Art Unit 1634		

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Application	Document	Mailroom Date	Attorney Docket No.
15634607	AP.PRE.DEC	08/10/2022	EXCTD-35239.304

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/634,607	06/27/2017	1634	1740	EXCTD-35239.304	19	2

CONFIRMATION NO. 1874

CORRECTED FILING RECEIPT

173254
CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

OC000000136099621

Date Mailed: 08/30/2022

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

Joost Louwagie, Dornach, SWITZERLAND;

Applicant(s)

Exact Sciences Corporation, Madison, WI;

Power of Attorney: The patent practitioners associated with Customer Number 72960

Domestic Priority data as claimed by applicant

This application is a CON of 15/010,436 01/29/2016 ABN
which is a CON of 13/147,570 03/12/2012 ABN
which is a 371 of PCT/GB2010/000180 02/03/2010
which claims benefit of 61/149,581 02/03/2009

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/12/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/634,607**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

To: DOCKETING@CASIMIRJONES.COM,Genomic_docketing@cardinal-ip.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 173254

Aug 30, 2022 03:23:11 AM

Dear PAIR Customer:

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 173254 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15634607	APP.FILE.REC	08/30/2022	EXCTD-35239.304

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	1874
Serial No.:	15/634,607	Group No.:	1634
Filed:	06/27/2017	Examiner:	Bradley L. Sisson
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

PETITION UNDER 37 CFR §1.182 TO REQUEST HANDLING OF THE APPLICATION PURSUANT TO M.P.E.P. § 707.02

Madam/Sir:

Applicant files this Petition under 37 CFR §1.182 to request that the application be recognized as "special" in accordance with M.P.E.P. §707.02, and to formally request the personal attention of the Supervisory Patent Examiner to any and all further actions in this application.

Section 707.02 of the M.P.E.P. directs that the Examiner's supervisor personally checks on the pendency of an application that is up for third action or in a 5-year application, with a view to finally concluding its prosecution, and further directs that, in order to accomplish this result, the application is to be considered "special." The above-captioned application was filed on **June 6, 2017**, more than 5 years ago, and is awaiting a **sixth action**, and thus falls within the directive of §707.02.

Substantive examination of the pending claims is summarized as follows:

1. Claims were entered in a Preliminary Amendment filed 10/13/2017; with non-elected claims canceled 05/22/2019 in response to a restriction requirement.

2. 1st Non-Final Office Action was mailed 06/27/2019; Applicant responded 9/27/2019.

3. 2nd Non-Final Office Action was mailed 01/21/2020; Applicant responded 5/22/2020.

4. 1st Final Office Action was mailed 08/10/2020. Applicant appealed, filing an Appellant's Brief on 03/09/2021.

5. 3rd Non-Final Office Action was mailed 06/28/2021, withdrawing finality of prior Office Action, and citing new art. Applicant responded 11/29/2021, amending claims.

6. 2nd Final Office Action was mailed 03/25/2022, citing new art. Applicant responded with an AFCP 2.0 request with after-final amendment 05/25/2022 (amendment not entered), then a Notice of Appeal with a Pre-Appeal Brief request for review, filed 06/27/2022.

7. A Notice of Panel Decision from Pre-Appeal Review was mailed on 08/10/2022, indicating that the finality of the 2nd Final Office Action has been withdrawn and prosecution is re-opened.

8. As of the date of this Petition filing, no new Office Action has been mailed.

Applicant notes that NO amendments were made to the elected claims between their filing on 10/13/2017 and the mailing of the 3rd Non-final Office Action on 06/28/2021. Thus, the same set of elected claims was before the Examiner for four of the Office Actions listed above.

Applicant now awaits a sixth Office Action on the merits in an application that is now more than 5 years old. Accordingly, Applicant requests that the application be recognized as "special" in accordance with M.P.E.P. §707.02, and requests the personal attention of the Supervisory Patent Examiner to any and all further actions in this application, as set forth in §707.02.

Applicant submits herewith the $420 petition fee set forth in § 1.17(f). Nevertheless, if the Director finds any other fee to be due in connection with this, or any other filing, authorization is given to charge the fees to Deposit Account No. 50-4302, referencing attorney docket number **EXCTD-35239.304**.

Dated: October 4, 2022 /Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
608.662.1277

Electronic Patent Application Fee Transmittal

Application Number:	15634607
Filing Date:	27-Jun-2017
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Filer:	Mary Ann Deese Brow
Attorney Docket Number:	EXCTD-35239.304

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
PETITION FEE- 37 CFR 1.17(F) (GROUP I)	1462	1	420	420
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**420**

Electronic Acknowledgement Receipt

EFS ID:	46751842
Application Number:	15634607
International Application Number:	
Confirmation Number:	1874
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
First Named Inventor/Applicant Name:	Joost Louwagie
Customer Number:	173254
Filer:	Mary Ann Deese Brow
Filer Authorized By:	
Attorney Docket Number:	EXCTD-35239.304
Receipt Date:	04-OCT-2022
Filing Date:	27-JUN-2017
Time Stamp:	15:16:40
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$420
RAM confirmation Number	E202204F18372972
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Petition for review by the Office of Petitions	2022-10-04_PetitionToDirector_EXCTD-003USCON2-35239_35239-304.pdf	114966 2da4ef6f807187bf3b3dc0c45e762cb7238f3237	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38024 c6bd01b1fc6cd40a2d9b6e7cbca94f13a39fc7c6	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	152990		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

173254 7590 12/02/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
WHISENANT, ETHAN C

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 12/02/2022

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	03/02/2023

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

173254 7590 12/02/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	03/02/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
WHISENANT, ETHAN C	1634	435-006110

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 12/02/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
WHISENANT, ETHAN C	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 12/02/2022

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/634,607	Applicant(s) Louwagie, Joost	
	Examiner Ethan C Whisenant	Art Unit 1634	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the paper(s) filed 10 AUG 2022.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634

Continuation of 3. The allowed claim(s) is/are: 82,84,87,89-94 and 96-100

NOTICE OF ALLOWABILITY

► **Claim(s) 82, 84, 87, 89-94 and 96-100** as amended in the paper(s) filed 10 AUG 2022 is/are pending in this application.

► The present application is being examined under the pre-AIA first to invent provisions. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

► Please note that this application has been transferred to a different examiner within Technology Center 1600, Art Unit 1634. See the closing paragraph of this action for details.

REASON(S) FOR ALLOWANCE

► **Claim(s) 82, 84, 87, 89-94 and 96-100** is/are deemed to be allowable in light of the paper(s) filed 10 AUG 2022 and the persuasive argument(s) therein. The Claims have now been renumbered as Claims 1-14 in the same order as presented by the applicant.

CONCLUSION

C. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen, can be reached at (571) 272-0731.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

EXAMINER SEARCH NOTES

21-22 NOV 2022 - ECW

Databases searched: All available in PE2E SEARCH
& CAplus, Medline and BIOSIS via STNext (note the search terms used below) ; and Google Scholar

Reviewed the parent(s), if any, and any search(es) performed therein : see the BIB data sheet

Reviewed, the search(es), if any, performed by prior examiners including any international examiners;
Reviewed the Search Report and Written Opinion in PCT/GB2010/000180

Search terms:

Inventor(s) e.g. Louwagie J?/au

Process$5

Feces or fecal

Sealable

Collection vessel or apparatus or device

Nuclease inhibitor?

Proteinase inhibitor?

► **See the Examiner's PE2E SEARCH notes/strategy in IFW**

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CPC						
Symbol					**Type**	**Version**
C12Q	/	1	/	6886	F	2013-01-01
G01N	/	33	/	57446	I	2013-01-01
C12Q	/	2600	/	106	A	2013-01-01
C12Q	/	2600	/	154	A	2013-01-01
C12Q	/	2600	/	158	A	2013-01-01
G01N	/	2800	/	50	A	2013-01-01
G01N	/	2800	/	52	A	2013-01-01
G01N	/	2333	/	805	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	22 November 2022	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office — Part of Paper No.: 20221121

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

INTERNATIONAL CLASSIFICATION				
CLAIMED				
C12Q1/6886	/	1	/	6886
G01N33/574	/	33	/	574

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 14	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634 (Primary Examiner)	22 November 2022 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

U.S. Patent and Trademark Office

Part of Paper No.: 20221121

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		14		27		40		53		66		79	7	92
	2		15		28		41		54		67		80	8	93
	3		16		29		42		55		68		81	9	94
	4		17		30		43		56		69	1	82		95
	5		18		31		44		57		70		83	10	96
	6		19		32		45		58		71	2	84	11	97
	7		20		33		46		59		72		85	12	98
	8		21		34		47		60		73		86	13	99
	9		22		35		48		61		74	3	87	14	100
	10		23		36		49		62		75		88		
	11		24		37		50		63		76	4	89		
	12		25		38		51		64		77	5	90		
	13		26		39		52		65		78	6	91		

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	14	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	22 November 2022	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office — Part of Paper No.: 20221121

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CPC - Searched*		
Symbol	**Date**	**Examiner**
C12Q 16886	11/22/2022	ECW
G01N 33/574	11/22/2022	ECW

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.
EAST	06/16/2021	B. L. S.
EAST	03/22/2022	B. L. S.
PE2E Search	06/09/2022	B. L. S.
See the Examiner's Search Notes on the last pg of the NOA	11/22/2022	ECW

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Ethan C Whisenant	1634

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
C12Q	1/6886	11/22/2022	ECW
G01N	33/574	11/22/2022	ECW

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	
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	5	-	-	-	-	-	-	-	-	
	6	-	-	-	-	-	-	-	-	
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	42	-	-	-	-	-	-	-	-	

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	
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	79	-	-	-	-	-	-	-	-	
	80	-	-	-	-	-	-	-	-	
	81	-	-	-	-	-	-	-	-	
1	82	÷	✓	✓	✓	✓	✓	✓	=	
	83	÷	✓	✓	✓	✓	-	-	-	
2	84	÷	✓	✓	✓	✓	✓	✓	=	
	85	÷	-	-	-	-	-	-	-	
	86	÷	-	-	-	-	-	-	-	
3	87	÷	✓	✓	✓	✓	✓	✓	=	
	88	÷	✓	✓	✓	✓	-	-	-	
4	89	÷	✓	✓	✓	✓	✓	✓	=	
5	90	÷	✓	✓	✓	✓	✓	✓	=	
6	91	÷	✓	✓	✓	✓	✓	✓	=	
7	92	÷	✓	✓	✓	✓	✓	✓	=	
8	93	÷	✓	✓	✓	✓	✓	✓	=	
9	94	÷	✓	✓	✓	✓	✓	✓	=	

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	
	95	÷	✓	✓	✓	✓	-	✓	-	
10	96	÷	✓	✓	✓	✓	✓	✓	=	
11	97	÷	✓	✓	✓	✓	✓	✓	=	
12	98	÷	✓	✓	✓	✓	✓	✓	=	
13	99	÷	✓	✓	✓	✓	✓	✓	=	
14	100	÷	✓	✓	✓	✓	✓	✓	=	

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L175	10223838	split OR divide	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:24 PM
L176	42026	174 AND 175	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:25 PM
L177	2933	174 SAME 175	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:25 PM
L178	14637	nuclease inhibitor	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU,	ADJ	ON	ON	2022/11/21 02:26 PM

			CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L179	0	177 SAME 178	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:26 PM
L180	8	177 AND 178	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:38 PM
L182	102661411	Process$	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN,	ADJ	ON	ON	2022/11/21 02:50 PM

			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L183	363700	feces OR fecal sample$	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:50 PM
L184	272390	182 AND 183	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:50 PM
L185	38178	182 AND 183 AND 175	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:51 PM
L186	738	182 SAME 183 SAME 175	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	ADJ	ON	ON	2022/11/21 02:51 PM

			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L187	0	182 SAME 183 SAME 175 AND 178	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:52 PM
L188	292	182 AND 183 AND 175 AND 178	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:52 PM
L189	516301	(proteinase OR protease) inhibitor	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT;	ADJ	ON	ON	2022/11/21 02:54 PM

			IBM_TDB)				
L190	195	188 AND 189	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/21 02:54 PM
L191	78984	protein marker	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/22 08:18 AM
L192	4812	183 AND 191	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/22 08:19 AM
L193	156	183 SAME 191	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	ADJ	ON	ON	2022/11/22 08:19 AM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L194	36	183 WITH 191	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/22 08:20 AM
L195	599	Louwagie.in.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/22 08:22 AM
L196	2	Louwagie.in. AND 193	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2022/11/22 08:22 AM
L197	52	Louwagie.in.	(US-PGPUB)	ADJ	ON	ON	2022/11/22

							08:24 AM
L198	10	Louwagie.in. AND 183	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:25 AM
L199	0	20170298448.AND 183	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:27 AM
L200	1	"20170298448." AND 183	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:27 AM
L201	1	"20170298448." AND 183 SAME 175	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:37 AM
L202	0	"20170298448." AND 183 SAME 175 AND L189 AND L178	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:39 AM
L203	0	"20170298448." AND 183 SAME 175 AND L189	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:39 AM
L204	0	"20170298448." AND 183 SAME 175 AND 178	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:40 AM
L205	1	"20170298448".pn. AND 183 SAME 175	(US-PGPUB)	ADJ	ON	ON	2022/11/22 08:51 AM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

To: DOCKETING@CASIMIRJONES.COM,Genomic_docketing@cardinal-ip.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 173254

Dec 02, 2022 03:24:28 AM

Dear PAIR Customer:

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 173254 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
15634607	NOA	12/02/2022	EXCTD-35239.304

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

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UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 12/06/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
WHISENANT, ETHAN C	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
12/06/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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Genomic_docketing@cardinal-ip.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
15/634,607	06/27/2017	Louwagie, Joost	EXCTD-35239.304

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
MARIANNE C SEIDEL	
ART UNIT	PAPER
1600	20221122

DATE MAILED: _____

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

This is in response to the Petition filed October 4, 2022 under 37 CFR §§ 1.182 and 37 CFR §§ 1.181, wherein applicants have requested that the application be recognized as "special" in accordance with M.P.E.P. §707.02, and to formally request the personal attention of the Supervisory Patent Examiner to any and all further actions in this application.

DECISION

This Petition is DISMISSED AS MOOT since the application has now been allowed.

Should there be any questions about this decision please contact Marianne C. Seidel by letter addressed to Director, TC 1600, at the address listed above, or by
telephone at 571-272-0584 or by facsimile sent to the general Office facsimile number, 571-273-8300.

/MC Seidel/
Marianne C. Seidel, Quality Assurance Specialist
Technology Center 1600

/MARIANNE C SEIDEL/
Quality Assurance Specialist, Art Unit 1600

PTO-90C (Rev.04-03)

To: DOCKETING@CASIMIRJONES.COM,Genomic_docketing@cardinal-ip.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 173254

Dec 07, 2022 03:26:34 AM

Dear PAIR Customer:

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 173254 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

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Application	Document	Mailroom Date	Attorney Docket No.
15634607	PETDEC	12/06/2022	EXCTD-35239.304

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

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Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PRINTER RUSH
(PTO ASSISTANCE)

Application: 15634607	**Examiner:** Whisenant	**GAU:** 1634
From:	**Location:** IDC	**Creation Date:** 12/06/2022

Week Date: 11/28/2022

DOC CODE	DOC DATE	MISCELLANEOUS
[ ] 1449		[ ] Continuing Data
[ ] IDS		[ ] Foreign Priority
[X] CLM	05/25/2022	[ ] Document Legibility
[ ] IIFW/FWCLM		[ ] Fees
[ ] SRFW		[ ] Petition (TC)
[ ] DRW		[X] Other
[ ] OATH		
[ ] 312		
[ ] SPEC		

[RUSH] Message:

Please clarify which claim set should be entered.
Latest claim set CLM 05/25/2022 has been stampped as "DO NOT ENTER" per ANE.I 06/14/2022.

Thank you.

[XRUSH] Response:

Initials: _____

Examiner: PUBS contact - James Leija, (703) 756-1197
NOTE: This form will be included as part of the official USPTO record with the response document coded as XRUSH.
REV: AUG 16

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 12/12/2022

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
WHISENANT, ETHAN C	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
12/12/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@CASIMIRJONES.COM
Genomic_docketing@cardinal-ip.com

Corrected ***Notice of Allowability***	**Application No.** 15/634,607	**Applicant(s)** Louwagie, Joost	
	Examiner Ethan C Whisenant	**Art Unit** 1634	**AIA (FITF) Status** No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the e-mail of 07 DEC 2022.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634

Continuation of 3. The allowed claim(s) is/are: 82,84,87,89-94 and 96-100

Corrected NOA

► In response to an e-mail inquiry regarding the status of the claims, the examiner is issuing this Corrected Notice of Allowability to clarify the record. The allowed Claims are : **Claims 82, 84, 87, 89-94 and 96-100** as presented in the paper(s) **filed 29 NOV 2021**. These Claims have now been renumbered as Claims 1-14 in the same order as presented by the applicant

► Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen, can be reached at (571) 272-0731.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	12/08/2022
	1	-	-	-	-	-	-	-	-	-
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	41	-	-	-	-	-	-	-	-	-
	42	-	-	-	-	-	-	-	-	-

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	12/08/2022
	43	-	-	-	-	-	-	-	-	-
	44	-	-	-	-	-	-	-	-	-
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	62	-	-	-	-	-	-	-	-	-
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	64	-	-	-	-	-	-	-	-	-
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	66	-	-	-	-	-	-	-	-	-
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	68	-	-	-	-	-	-	-	-	-
	69	-	-	-	-	-	-	-	-	-
	70	-	-	-	-	-	-	-	-	-
	71	-	-	-	-	-	-	-	-	-
	72	-	-	-	-	-	-	-	-	-
	73	-	-	-	-	-	-	-	-	-
	74	-	-	-	-	-	-	-	-	-
	75	-	-	-	-	-	-	-	-	-
	76	-	-	-	-	-	-	-	-	-
	77	-	-	-	-	-	-	-	-	-
	78	-	-	-	-	-	-	-	-	-
	79	-	-	-	-	-	-	-	-	-
	80	-	-	-	-	-	-	-	-	-
	81	-	-	-	-	-	-	-	-	-
1	82	÷	✓	✓	✓	✓	✓	✓	=	=
	83	÷	✓	✓	✓	✓	-	-	-	-
2	84	÷	✓	✓	✓	✓	✓	✓	=	=
	85	÷	-	-	-	-	-	-	-	-
	86	÷	-	-	-	-	-	-	-	-
3	87	÷	✓	✓	✓	✓	✓	✓	=	=
	88	÷	✓	✓	✓	✓	-	-	-	-
4	89	÷	✓	✓	✓	✓	✓	✓	=	=
5	90	÷	✓	✓	✓	✓	✓	✓	=	=
6	91	÷	✓	✓	✓	✓	✓	✓	=	=
7	92	÷	✓	✓	✓	✓	✓	✓	=	=
8	93	÷	✓	✓	✓	✓	✓	✓	=	=
9	94	÷	✓	✓	✓	✓	✓	✓	=	=

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/18/2019	06/23/2019	01/15/2020	08/04/2020	06/16/2021	03/22/2022	06/09/2022	11/22/2022	12/08/2022
	95	÷	✓	✓	✓	✓	-	✓	-	-
10	96	÷	✓	✓	✓	✓	✓	✓	=	=
11	97	÷	✓	✓	✓	✓	✓	✓	=	=
12	98	÷	✓	✓	✓	✓	✓	✓	=	=
13	99	÷	✓	✓	✓	✓	✓	✓	=	=
14	100	÷	✓	✓	✓	✓	✓	✓	=	=

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(Submitted Only via EFS-Web)

Application Number	15/634,607	Filing Date	2017-06-27	Docket Number (if applicable)	EXCTD-35239.304	Art Unit	1634
First Named Inventor	JOOST LOUWAGIE			Examiner Name	WHISENANT		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
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Signature	/Mary Ann D. Brow/	Date (YYYY-MM-DD)	2023-02-06
Name	Mary Ann D. Brow	Registration Number	42363

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(54) Title: METHODS FOR PRESERVING DNA INTEGRITY

(57) Abstract

Methods for extracting DNA from a biological sample that result in a higher yield of target DNA than conventional methods. More particularly, methods for extracting DNA include exposing the biological sample to inhibitors of DNA degradation.

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Methods for Preserving DNA Integrity

Cross-Reference to Related Application

The present application claims priority to and the benefit of U.S. provisional patent application serial number 60/122,177, filed February 25, 1999, the entire disclosure of which is incorporated herein by reference.

Technical Field

The invention provides methods for deoxyribonucleic acid ("DNA") extraction from a biological sample. More particularly, the invention relates to methods for high yield DNA extraction from a heterogeneous biological sample by inhibiting DNA degradation.

Background of the Invention

DNA is a relatively stable molecule that is routinely isolated from biological samples. Recently, many diseases involving instabilities (*e.g.*, mutations) in genomic DNA have been characterized. Also, many pathogens have been identified by the presence or absence of a particular DNA in a biological sample. Many diseases, such as cancer, are optimally detected early in their progression. In order for early detection to be effective, relatively low levels of DNA which are indicative of cancer must be detected against a high background of other DNA (*e.g.*, normal human DNA, bacterial DNA, etc.). This type of detection is technically difficult and typically results in low sensitivity of detection. Moreover, in certain complex specimens, including stool, what little species-specific DNA exists, is rapidly degraded, making efficient sequence-specific detection even more difficult. Thus, a need exists for methods to retain integrity of DNA in a sample, especially in samples in which the DNA to be detected is in low proportion relative to other DNA in the sample and is degraded quickly.

Summary of the Invention

The present invention provides methods for preserving the integrity of DNA in a sample. In a preferred embodiment, methods of the invention prevent enzyme-mediated DNA degradation. Preservation of DNA integrity facilitates isolation and detection of DNA.

Methods of the invention are especially useful for extracting or detecting DNA in a biological specimen, especially one that contains low levels of relevant DNA. A good example of a specimen that contains lower-levels of relevant DNA is stool. Typical human stool contains only small amounts of intact human DNA. Most of the human DNA in stool from a healthy individual is presumably from exfoliated epithelial cells, and has undergone apoptotic degradation. As the forming stool passes through the colon, colonic epithelial cells are sloughed onto the stool as part of the cellular turnover that occurs in the colon. Stool also contains sloughed cells from other luminal sources (*e.g.*, lung, stomach, esophagus, *etc.*) Sloughed cells typically have undergone or are undergoing apoptosis, leaving cellular DNA in small fragments. Enzymes, such as deoxyribonuclease ("DNase") and Micrococcal nuclease contribute to the degradation of any intact human DNA that remains. Prior art methods, while using DNase inhibitors, have failed to achieve significant yields of intact, species-specific DNA from stool. Therefore, such methods failed to consider optimization of inhibition of DNA degradation. Methods of the invention are based on the realization that optimal inhibition of DNA degrading enzyme(s) effectively preserves DNA, especially large, diagnostically-relevant DNA fragments that are present in a sample.

In one aspect, the invention comprises inhibiting nucleic acid degradation in a sample and optionally extracting a target DNA with, for example, a phenol-chloroform extraction. Preferably, the inhibition of nucleic acid degradation is sufficient to produce a critical number of

molecules of analyzable DNA. In one embodiment, methods of the invention comprise inhibiting an enzyme capable of DNA degradation in a stool sample. In a preferred embodiment, methods of the invention comprise exposing a stool sample to an ion chelator, such as a divalent ion chelator. Ion chelators, in certain embodiments inhibit DNase. Examples of preferred inhibitors include ethylenediaminetetraacetic acid ("EDTA"). Additional preferred methods of the invention comprise exposing a stool sample to a Micrococcal nuclease inhibitor, such as EGTA, also a divalent ion chelator. Inhibitors of DNA degradation may be used either alone or in combination to achieve optimal levels of DNA preservation.

Methods of the invention are practiced using any inhibitor of DNA degradation. The amount of inhibitor varies depending on the inhibitor that is used. However, an inhibitor must be used in an amount that preserves significant levels of DNA in the sample for subsequent analysis. Methods for determining sufficient levels of DNA are presented below. Such methods allow the skilled artisan to practice the invention with specificity regardless of the inhibitor used. According to preferred methods, an amount of inhibitor is used that preserves sufficient DNA in the sample for detection of a target DNA within a desired level of statistical confidence. Using methods described herein, the skilled artisan can determine an appropriate amount of any inhibitor for use in methods of the invention. The use of various specific inhibitors is exemplified below.

In another preferred embodiment, methods of the invention comprise obtaining a representative (circumfrential or cross-sectional) stool sample, exposing the sample or a portion thereof to a DNase inhibitor, and isolating DNA from the sample. One preferred DNase inhibitor is EDTA. Preferred amounts of EDTA are from about 0.042 g per gram of stool to about 0.782 g per gram of stool and especially from about 0.250 g per gram of stool to about

0.521 g per gram of stool. DNA may be extracted, for example, by a phenol-chloroform extraction. After extraction, the DNA may be analyzed by methods known in the art. For example, U.S. Patent No. 5,830,665 and U.S. Patent No. 5,670,325, which are incorporated by reference herein, disclose methods for analyzing DNA which has been extracted from a stool sample.

Methods of the invention are useful in any sample in which inhibition of DNA degradation is desired. For example, methods of the invention are especially effective in samples comprising exfoliated cells, especially exfoliated epithelial cells. The DNA contained in such samples typically degrades rapidly, making analysis of a particular DNA, especially one that exists in low proportion within the sample, difficult. For example, such samples include stool, sputum, urine, pus, and collostrum. Methods of the invention include inhibiting DNA degradation in such samples, thus preserving a sufficient amount of DNA for specific, sensitive detection. Any of the features described above, such as DNA degradation inhibitors or amounts of inhibitors that are used, can be useful in samples containing exfoliated cells.

Brief Description of the Drawings

FIG. 1 shows a flow chart describing one aspect of the invention.

FIG. 2 shows a separation gel of DNA isolated from several different homogenized stool supernatants that contained various concentrations of EDTA.

FIG. 3 shows a separation gel of DNA isolated from homogenized stool supernatant that contained various concentrations of EDTA followed by capture and amplification.

FIG. 4 shows separation gel of DNA isolated from homogenized stool supernatant that contained various concentrations EDTA followed by capture, addition of more DNA, and amplification.

FIG. 5 shows a set of curves produced by regression analysis of the data obtained using the model as described for, for example, Tables 1 and 2.

Detailed Description of the Invention

I. Introduction

The present invention provides methods for increased yield of DNA in a biological sample by preserving the integrity of DNA in the sample. Such methods are especially useful when the DNA of interest ("the target DNA") is present in the sample at a low frequency, or is rapidly degraded. More particularly, methods of the invention include, for example, inhibiting enzymes that degrade DNA.

Prior to the present invention, those skilled in the art have not been concerned with preventing DNA degradation prior to extraction from a sample. Typically, either the DNA of interest is present in samples in relatively large quantities (*e.g.*, tumor cells, blood), or methods are directed toward increasing sensitivity to low-frequency DNA, and not to preserving its integrity. However, especially in the case of low-frequency DNA in a heterogeneous sample (*e.g.*, a sample having cells and/or cellular debris from multiple cell types and/or organisms), methods for increasing sensitivity to DNA have not been entirely successful. Methods of the invention provide a new approach by preserving the integrity of DNA. Methods of the invention increase the likelihood of detecting a specific target DNA, because such methods make more intact target DNA available in the sample.

Referring to Figure 1, one generalized method of the invention involves obtaining a sample (step 2) and exposing it to a DNA degradation inhibitor (step 4). Once the sample has been exposed to the DNA degradation inhibitor, target DNA is extracted (step 6). The presence or absence of this extracted target DNA is then detected (step 8).

In heterogeneous samples, such as stool, endogenous human DNases and/or bacterial nucleases degrade DNA. Examples of nucleases include DNases, such as deoxyribonuclease I ("DNase I") and Micrococcal nuclease. DNase and Micrococcal nuclease both require a divalent cation to function optimally. For DNase, suitable ions include Mn^{+2} and Mg^{+2}. For Micrococcal nuclease, Ca^{+2} is a suitable ion. Ion chelators, and particularly divalent ion chelators, are capable of inhibiting nucleases. Ion chelators remove ions from association with the nuclease, thus inhibiting the nuclease's function. For example, EDTA or EGTA used in optimal amounts are useful ion chelators for use in the present invention.

Other compounds that inactivate, interfere with, or slow enzyme-mediated degradation of DNA are useful. For example, ligands and/or antibodies which compete for or interfere with the active site of DNA degrading enzymes, which inactivate those enzymes, and/or which block messenger systems that control DNA degrading enzymes are useful in the practice of the invention. Phenol-chloroform extraction components, at higher concentrations than those typically used during extraction, also are capable of inhibiting nucleases by separation and denaturation. For example, phenol denatures DNA degradation enzymes, and is used in methods of the invention to preserve DNA integrity. Also, proteinases which degrade and/or denature DNA degrading enzymes are useful.

Methods of the invention comprise the use of optimal amounts of DNA degradation inhibitors in order to preserve high-integrity DNA sufficient for diagnostic screening. In

heterogeneous samples, such as stool, target DNA (*e.g.*, mutated DNA or reductions in enumerated wild type DNA that are indicative of a mutation) is present in low amounts. An optimal amount of a DNA degradation inhibitor is an amount that results in a measurable improvement in the quantity of DNA available in the sample. Thus, the skilled artisan can empirically determine optimal amounts of DNA degradation inhibitors for use in methods of the invention by using inhibitor amounts necessary to preserve a diagnostically-relevant fraction of high-integrity target DNA. A method for determining diagnostically-relevant DNA amounts is presented below.

Amplification of DNA, and other stochastic processes, performed on heterogeneous samples may actually contribute to the inability to measure low-frequency DNA. For example, a typical cancer-associated (mutant) DNA in the early stages of oncogenesis represents about 1% of the DNA in a heterogeneous sample (*e.g.*, stool). If DNA in the sample is amplified at 30% PCR efficiency, any particular DNA has only a 30% chance of being amplified in any round of PCR. Thus, if a mutant DNA initially present as 1% of a sample is not amplified in the first round, the mutant DNA will represent only about 0.7% of the DNA in the sample after round 1. If no mutant is amplified in the first two rounds (0.7 x 0.7, or a 49% probability), the mutant DNA will represent only about 0.6% of the DNA in the sample going into round three of the PCR . If the post-amplification assay used to detect the mutant has a sensitivity of no more than 0.5% for the mutant, it may not be possible to reliably detect the presence of the mutant DNA. Thus, the detection method itself may actually contribute to difficulties in detecting low-frequency DNA, especially if sufficient amounts of intact DNA are not present in a sample. Thus, one means for determining an appropriate amount of inhibitor to use in methods of the invention is to determine the minimum amount of intact DNA that must be present in a sample to

avoid the stochastic effects described above, and then to use sufficient inhibitor to produce at least the minimum number of DNA molecules in the sample. Methods for calculating the minimum number of DNA molecules necessary to overcome the effects of stochastic processes, such as PCR, are presented below.

A model useful to generate sufficient DNA molecules for accurate measurement operates by iterating stochastic processes over a number of rounds of PCR. In the context of molecular disease diagnostics, the model dictates the number of molecules that must be presented to the PCR in order to reliably ensure amplification of desired target DNA. The model incorporates a preset PCR efficiency (established to meet separate specificity requirements), and a preset ratio of mutant DNA to total DNA in the sample to be analyzed (which is a property of the disease to be detected and the nature of the sample). Based upon those input values, the model predicts the number of molecules that must be presented to the PCR in order to ensure, within a predefined level of statistical confidence, that a low-frequency (target) molecule will be amplified and detected. Once the number of molecules is determined, the skilled artisan can determine the sample size to be used (*e.g.*, the weight, volume, etc.), depending on the characteristics of the sample (*e.g.*, its source, molecular makeup, etc.). The model dictates the number of molecules that must be presented to the PCR in order to reliably ensure amplification and detection.

The exemplary model simulates selection of DNA for amplification through several rounds of PCR. For purposes of the model, a sample is chosen that contains a ratio of mutant-to-total DNA of 1:100, which is assumed to lie at the clinical threshold for disease. For example, in colorectal cancer 1% of the human DNA in a specimen (*e.g.*, stool) is mutated (*i.e.*, has a deletion, substitution, rearrangement, inversion, or other sequence that is different than a corresponding wild-type sequence). Over a large number of PCR rounds, both the mutant and

wild-type molecules will be selected (*i.e.*, amplified) according to their ratio in the specimen (here, nominally 1 in 100), assuming there are any abnormal molecules in the sample. However, in any one round, the number of each species that is amplified is determined according to a Poisson distribution. Over many rounds, the process is subject to stochastic errors that reduce the ability to detect low-frequency mutant DNA. However, the earlier rounds of PCR (principally, the first two rounds) are proportionately more important when a low-frequency species is to be detected, and any rounds after round 10 are virtually unimportant. Thus, the model determines the combined probability of (1) sufficient mutant molecules being presented to the PCR, and (2) the effects of stochastic amplification on those molecules so that at the output of the PCR there will be a sufficient number of molecules and a sufficient ratio of mutant to total molecules to assure reliable detection.

The model used to run the number of molecules necessary at the first round of PCR was generated as a "Monte Carlo" simulation of a thousand experiments, each experiment consisting of 10 cycles of PCR operating on each molecule in the sample. The simulation analyzed (1) taking a sample from the specimen; and (2) each round of PCR iteratively to determine whether, for each round, a mutant DNA if present in the sample was amplified. Upon completion of the iterative sampling, the model determined the percent of rounds in which a mutant strand was amplified, the percent of mutants exceeding a predetermined threshold for detection (in this example 0.5% based upon the mutant:total ratio of 1%), the coefficient of variation (CV) for stochastic sampling in each round alone, and the coefficient of variance for stochastic sampling and PCR in combination.

Stochastic noise is created in PCR if the PCR efficiency is anything other than 0% or 100% (these two cases represent either there is no amplification at all or perfect fidelity of

specific amplification). The noise, or background, signal level in a PCR that is between 0% and 100% varies with the efficiency of the PCR. The standard deviation of stochastic noise, S, in a PCR is given by the equation, $S = \sqrt{npq}$, where n is the number of molecules in the sample, p is the efficiency of PCR, and q is 1-p. Table 1 presents results obtained for iterative samplings with PCR efficiency set at 100% and 20%, and a mutant:total ratio of 0.5%.

Table 1 represents output from the model in 12 experiments conducted under various conditions. The first row shows the nominal number of molecules entering the first round of PCR (i.e., the total number of molecules available for amplification). The second row shows the percent of molecules (DNA) in the biological specimen that is expected to be mutant. For colorectal cancer indicia in DNA recovered from stool, the threshold for clinical relevance in the detection of early stage cancer is 1%. That is, 1% of the DNA in a sample derived from a heterogeneous specimen (e.g., stool) contains a mutation associated with colorectal cancer. The 6th row is the threshold of detection of the assay used to measure PCR product after completion of PCR. That number is significant, as will be seen below, because sufficient mutant DNA must be produced by PCR to be detectable over aberrant signal from wild-type and random background noise. Under the heading "Outputs", the first line provides the likelihood that at least one mutant molecule is presented to the first round of PCR. The second line under the Output heading provides the likelihood of detection of mutants (after PCR) above the predetermined threshold for detection. For example, in experiment 4, the results indicate that in 87.9% of experiments run under the conditions specified for experiment 4, the number of mutants will exceed the threshold number for detection. Finally, the last two rows provide the coefficient of variation for sampling, and for the combination of sampling and PCR.

TABLE 1

	100% Efficiency PCR						20% Efficiency PCR					
Inputs	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Exp 9	Exp 10	Exp 11	Exp 12
Nominal number of Molecules going into PCR	50	100	200	500	1,000	10,000	50	100	200	500	1,000	10,000
Percent of molecules that are mutant	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
Number of PCR Rounds	10	10	10	10	10	10	10	10	10	10	10	10
Efficiency of PCR per round	100%	100%	100%	100%	100%	100%	20%	20%	20%	20%	20%	20%
Number of experiments modeled	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
M:W ratio which can be reliably detected by assay	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
Outputs												
% of Experiments each with the number of mutant cells > 0	39.30%	64.40%	87.30%	99.40%	100%	100%	39.10%	63.50%	86.10%	99.60%	100%	100%
% of Experiments each with number of mutant cells exceeding threshold	39.30%	64.40%	87.30%	87.90%	97.10%	100%	36.20%	52.40%	63.30%	63.30%	91.60%	100%
Stochastic Sampling CV	143.20%	100%	70%	44.10%	32.10%	9.90%	144.60%	101.80%	72.00%	44.20%	32.00%	10%
Stochastic Sampling and Stochastic PCR CV	143.20%	100%	70%	44.10%	32.10%	9.90%	179.70%	123.10%	92.10%	56.40%	40.30%	12.70%

As shown in Table 1, even at 100% PCR efficiency, mutant DNA is detected in only 97.1% of the samples when 1000 input molecules are used (*i.e.*, 1000 DNA molecules are available for priming at the initial PCR cycle), even though 100% of the DNA is amplified in any given round of PCR. When 10,000 molecules are presented, it is virtually certain that the mutant DNA will be amplified and detected, as shown in the results for experiment 6 in Table 1. Stochastic errors due to variation in the number of input molecules become less significant at about 500 input molecules and higher (*i.e.*, the CV for stochastic variations is about the same regardless of whether PCR efficiency is 20% or 100%). At lower PCR efficiency (20% in Table 1), the model shows that introducing 50, 100, 200, 500, or even 1000 molecules into the PCR does not assure either amplification or detection. As shown in experiment 12, introducing 10,000 molecules results in amplification of the mutant target, and a high likelihood of its subsequent detection. Thus, even with 100% efficient PCR, significant false negative events occur when input molecules fall below 500.

The foregoing analysis shows that there is a unique range for the number of molecules that must be presented to a PCR in order to achieve amplification of a low-frequency DNA, and to allow its detection. That range is a function of the PCR efficiency, and the percentage of low-frequency (mutant) DNA in the sample, and the detection threshold. The aforementioned model was developed and run in Visual Basic for Applications code (Microsoft, Office 97) to simulate a PCR as described above. The statistical confidence level within which results were measured was held constant at approximately 99%. Only the PCR efficiency and percent mutant DNA were varied. As discussed above, the model iteratively samples DNA in a "Monte Carlo" simulation over a thousand experiments, each experiment consisting of 10 rounds of PCR. The results are shown below in Table 2.

TABLE 2

	Number of molecules needed			
PCR Efficiency	1% Mutant	2% Mutant	5% Mutant	10% Mutant
10%	3,000			
20%	2,500			
50%	2,200			
100%	1,600			
10%		1,500		
20%		1,200		
50%		1,000		
100%		800		
10%			500	
20%			450	
50%			400	
100%			300	
10%				225
20%				200
50%				150
100%				125

Regression of the data obtained using the model as described above produced the set of curves set forth below in Figure 5.

Using Figure 5, the optimal number of molecules to be presented to the PCR is determined by selecting a PCR efficiency (or determining the efficiency by empirical means), and selecting a percentage of the sample suspected to be mutant DNA associated with disease. This, in turn, dictates a threshold of detection. Not all detection strategies have similar underlying detection thresholds, so an appropriate technology must be selected. The percentage mutant DNA may be determined by clinical considerations as outlined above for colorectal cancer.

One may determine the PCR efficiency and percent expected mutant in order to maximize the probability of obtaining amplified, detectable mutant DNA. For example, one may select N, the number of input molecules from the "1%" curve in Figure 5, when 5% of the

sample is expected to be mutant DNA in order to increase the confidence of the assay result.

This model, and particularly Figure 5, are useful when determining optimal concentrations of a DNA degradation inhibitor. If PCR is used to analyze DNA after the DNA sample is exposed to a DNA inhibitor, Figure 5, will indicate how many molecules of target DNA need to be preserved in order to have sufficient analyzable DNA. Thus, the optimal amount of any DNA degradation inhibitor may be determined as that amount, or range of amounts, of inhibitor that produce a sufficient number of analyzable DNA according to Figure 5. Of course, this modeling system may be applied to DNA detection techniques other than PCR. Specifically, those skilled in the art can apply this modeling system to any process and/or detection technique in which stochastic noise is problematic. Thus, the optimal amount of any DNA degradation inhibitor can be determined based upon the number of DNA molecules that are sufficient to produce analyzable DNA.

Once the number of molecules for input to the PCR is determined, a sample comprising that number of molecules (or greater) is prepared for PCR according to standard methods. The number of molecules in a sample may be determined directly by, for example, enumerative methods such as those taught in U.S. Patent No. 5,670,325, incorporated by reference herein. Alternatively, the number of molecules in a complex sample may be determined by molar concentration, molecular weight, or by other means known in the art. The amount of DNA in a sample may be determined by mass spectrometry, optical density, or other means known in the art. The number of molecules in a sample derived from a biological specimen may be determined by numerous means in the art, including those disclosed in U.S. Patent Nos. 5,741,650 and 5,670,325, both of which are incorporated by reference herein.

Methods as described above are used to determine minimum or optimal amounts of DNA

degradation inhibitors for use in any DNA isolation, detection, or amplification process in which stochastic processes occur. Using the above-described model for determining the minimum number of molecules that must be measured to reliably detect a low-frequency species, one can empirically determine how much of any given inhibitor should be used.

II. Example - Detection of DNA in Stool with EDTA as a DNA Degradation Inhibitor

A. Introduction

Methods of the invention are useful for analyzing DNA from stool to detect colorectal cancer. If colorectal cancer is diagnosed early, it may be treated effectively by surgical removal of the cancerous tissue. Colorectal cancers originate in the colorectal epithelium, and typically are not extensively vascularized (and therefore not invasive) during the early stages of development. The transition to a highly vascularized, invasive and ultimately metastatic cancer which spreads throughout the body commonly takes ten years or longer. If the cancer is detected prior to invasion, surgical removal of the cancerous tissue is an effective cure. However, colorectal cancer is often detected only upon manifestation of clinical symptoms, such as pain and black tarry stool. Generally, such symptoms are present only when the disease is well established, often after metastasis has occurred, and the prognosis for the patient is poor, even after surgical resection of the cancerous tissue. Early detection of colorectal cancer, therefore, is important because early detection may significantly reduce patient morbidity.

B. Experiments

The following experiments demonstrate that EDTA, an inhibitor of DNase, increases the yield of high-integrity DNA from a stool sample with a concomitant increase in the amount of amplifiable DNA. In these experiments, three aliquots of stool (5 g each) were homogenized in buffer (0.5 M Tris, 10 mM NaCl, EDTA). The buffer to stool ratio was 7:1; thus, 35 ml of buffer

was used for each 5 g of stool. The buffer contained either 0 mM EDTA, 16 mM EDTA, or 96 mM EDTA. Each of the three aliquots was then diluted with additional buffer (not containing EDTA) to a final buffer to stool ratio of 20:1. Each aliquot was then centrifuged, and the supernatant, which carried the active DNA degrading fraction, was removed to a clean tube. Then, a DNA mixture of 2 μg *E. coli* DNA and 100 ng human genomic DNA was added to each tube. Each tube was incubated for 75 minutes at 37°C. Then, 42 μl of Proteinase K and 250 μl of 10% SDS (sodium dodecyl sulfate) were added to each tube followed by an overnight incubation at 37°C. After the overnight incubation, the DNA in each sample was prepared by standard techniques. *See, e.g.*, SHORT PROTOCOLS IN MOLECULAR BIOLOGY §§ 2.1-2.4 (Ausubel *et al.*, 3d ed., 1995). Generally, a phenol extraction, a phenol/chloroform extraction, and a phenol extraction were performed prior to isolating the DNA. Then, the isolated DNA was placed into a standard Tris buffer.

Three experiments were conducted on the isolated DNA. The first experiment demonstrated that the DNA degrading activity present in homogenized stool supernatant is inhibited by optimal amounts of EDTA, increasing the amount of high-integrity DNA. In this experiment, DNA was isolated from homogenized stool supernatant which was taken from aliquots of stool homogenized in buffer having 0 mM, 16 mM, or 96 mM EDTA. Total nucleic acid was run on a separation gel. Results are shown in Figure 2, where arrows identify the location of the smear (or lack thereof) containing the DNA of interest.

Lanes 4, 5, and 6 represent samples of DNA added to homogenized stool supernatant, obtained from stool homogenized in buffers containing 0 mM, 16 mM, or 96 mM EDTA, respectively, that was subsequently isolated. Note that each lane shows a high molecular weight band which represents endogenous DNA from the stool sample and a smear from the exogenous

DNA. The intensity of the band and smear in the photograph (which correlates with the amount of DNA in the band, a greater intensity corresponding to a greater amount of DNA) increased as the concentration of EDTA in the original buffer increased. Lanes 7-9 and 10-12 are replicates of lanes 4-6. The increasing intensity of the bands and the smears as EDTA concentration increased indicated that DNA integrity was preserved as the concentration of EDTA in the buffer increased. Thus, the DNA degrading activity of the homogenized stool supernatant was inhibited by the EDTA in a roughly dose-dependent manner.

Lanes 1, 2 and 3 and lanes 13, 14 and 15 were control samples containing 2 μg *E. coli* DNA and 100 ng exogenous human DNA in buffer made with 16 mM EDTA. As expected, each lane showed a smear representative of the added DNA.

In a second experiment, the isolated DNA as described above was captured and amplified. The results of this experiment demonstrated that EDTA not only inhibits the DNA degrading activity present in stool supernatant but also increases the amount of amplifiable DNA. In this experiment, after preparing the DNA as described above, a standard hybrid capture was performed using Kras-specific capture probes to capture Kras DNA. The Kras DNA then was PCR amplified. Figure 3 shows the effect of EDTA on the preservation of Kras DNA. The location of the band (or lack thereof) representing Kras DNA is identified with an arrow.

Lanes 4, 5, and 6 represent Kras DNA that was amplified from template DNA that was added to homogenized stool supernatant obtained from stool homogenized in buffer containing 0 mM, 16 mM, or 96 mM EDTA, respectively. Note that the Kras band in lane 4 was nearly absent, while the Kras band grew in intensity (representing an increase in the amount of Kras DNA actually present) in lanes 5 and 6 as the concentration of EDTA in the buffer increased. Lanes 7-9 are replicates of lanes 4-6 and show a similar increase in band intensity (an increase in

the amount of DNA present) as the concentration of EDTA in the original buffer increased. Thus, more Kras DNA was amplified, resulting in a more robust signal at higher concentrations of EDTA.

Additionally, in the population, levels of DNA which can be amplified from stool vary across individuals. These individuals have been characterized in groups from A to F, with A being the highest level of DNA and F being an undetectable level of DNA. The high levels of DNA in group A are due to low DNA degradation activity in their stool ("high-integrity stool"). Adding EDTA to the buffer in which a stool aliquot from a Group A individual is homogenized would not be expected to produce a large effect because Group A stool has little DNA degrading activity. In fact, when Kras DNA was amplified from template DNA that was added to homogenized stool supernatant obtained from Group A stool homogenized in buffer containing 0 mM, 16 mM, or 96 mM EDTA, little difference in the amount of amplified Kras DNA was observed (lanes 10, 11, and 12, respectively). Only a slight increase in Kras band intensity can be seen between 0 mM EDTA and 16 mM or 96 mM EDTA, representing only a slight increase in the amount of Kras DNA at inhibitory concentrations of EDTA.

Lanes 1-3 as well as lanes 13-15 represent samples of amplified Kras DNA that was not exposed to homogenized stool supernatant. As expected, those control lanes show a band of equal intensity across lanes representing Kras DNA. Lanes 16 and 17 are negative controls and, as expected, show no band representing Kras, indicating that any observed Kras DNA is due to captured DNA and not to contamination. Lane 18 is a negative control, and, as expected, has no band representing the Kras gene, indicating that the PCR products are from the sample and not from contamination. Lanes 19-21 are positive controls where 50 pg, 100 pg, or 200 pg of human

DNA is amplified in a background of *E. coli* DNA, indicating that human DNA can be amplified in an *E. coli* background in this model system. Finally, lane 22 is a molecular weight marker.

In a third experiment, the same protocol was used as in the second experiment except human genomic DNA was added into each sample after capture. Kras DNA was again amplified by PCR. Thus, an excess of template DNA was available for PCR. This experiment demonstrated that the varying levels of PCR amplification in the second experiment were not due to EDTA interfering with or enhancing normal PCR but were due to varying levels of template DNA available to be amplified resulting from various levels of DNA degradation inhibition by EDTA. Figure 4 shows the results of this experiment. The location of the band (or lack thereof) representing Kras is identified with an arrow. Lanes 1-6 and 8-13 correspond with lanes 1-12 in the second experiment (*i.e.*, lanes 1-3 were controls, lanes 4-6 and 8-10 were excess Kras DNA amplified in samples exposed to homogenized stool supernatant from stool homogenized in 0 mM, 16 mM, or 96 mM EDTA, and lanes 11-13 were excess Kras DNA amplified in samples exposed to homogenized stool supernatant from high-integrity stool homogenized in 0 mM, 16 mM, or 96 mM EDTA). As expected, lanes 1-6 and 8-13 show a PCR product of roughly equal intensity because an excess of template DNA is available. The EDTA does not interfere with or enhance normal PCR. This result indicates that the varying levels of PCR amplification in the 0 mM, 16 mM, or 96 mM EDTA samples in the second experiment was due to varying levels of template DNA and not to inhibitor. Lane 14 shows a sample of human genomic DNA. Lanes 15-18 are the same controls as lanes 17 and 19-21 in the second experiment.

From the experimental data described above, the amount of EDTA required to inhibit DNase was calculated. The concentration of EDTA in the various buffers used in the three

experiments was normalized as grams of EDTA per gram of stool. Generally, the concentration of EDTA was multiplied by the molecular weight of EDTA and by the volume of buffer in which the stool was homogenized. The product was divided by the amount of stool that was homogenized. For example, the following equations were used to normalize EDTA concentration.

For 16 mM EDTA:

(0.016 EDTA M/L x 372.2 g/M x 0.035L) ÷ 5g = 0.042 g EDTA per gram of stool

For 96 mM EDTA:

(0.096 EDTA M/L x 372.2 g/M x 0.035L) ÷ 5g = 0.250 g EDTA per gram of stool

Thus, for any amount of stool to be homogenized, at least about 0.042 g EDTA per gram of stool should be used in the homogenization buffer in order to maximize yield of DNA. The range of EDTA which may be used is from about 0.042 g EDTA per gram of stool to about 0.782 g EDTA per gram of stool. More preferably, about 0.250 g EDTA per gram of stool to about 0.521 g EDTA per gram of stool is used. Most preferably, about 0.391 g EDTA per gram of stool is used.

These calculations indicate that at commonly used buffer volumes and stool amounts, the amount of EDTA present in the homogenized sample is a more important factor than the final concentration of EDTA in the homogenized sample. However, as one skilled in the art realizes, at some point, although the amount of EDTA will remain the same in a given volume, the volume may become so large that the effect of EDTA on DNA integrity is diluted. When examining a stool sample within commonly used parameters, this dilution effect is not seen. However, in alternative embodiments, the concentration of EDTA is a relevant factor. In these embodiments, from about 16 mM EDTA to about 300 mM EDTA is useful. More preferably,

from about 100 mM EDTA to about 200 mM EDTA is useful. Most preferably, about 150 mM EDTA is useful.

What is claimed is:

1. A method for preserving the integrity of DNA in a stool sample, the method comprising:

exposing the stool sample to an amount of an inhibitor of DNA degradation sufficient to produce a critical number of molecules of analyzable DNA.

2. The method of claim 1 wherein the inhibitor comprises an ion chelator.

3. The method of claim 2 wherein the ion chelator comprises a divalent ion chelator.

4. The method of claim 3 wherein the divalent ion chelator is selected from the group consisting of EDTA and EGTA.

5. The method of claim 1 wherein the step of exposing the stool sample to an amount of an inhibitor of DNA degradation comprises providing EDTA in a range from 0.042 g EDTA per gram of stool to 0.782 g EDTA per gram of stool.

6. The method of claim 1 wherein the inhibitor of DNA degradation comprises an enzyme inhibitor.

7. The method of claim 1 further comprising a step of extracting a target DNA.

8. The method of claim 7 wherein the step of extracting comprises a phenol-chloroform extraction.

9. A method for preserving the integrity of DNA in a sample containing exfoliated cells, the method comprising:

exposing the sample to a minimum amount of an inhibitor of DNA degradation sufficient to produce a critical number of molecules of analyzable DNA.

10. The method of claim 9 wherein the sample is obtained from stool.

11. The method of claim 9 wherein the inhibitor comprises an ion chelator.

12. The method of claim 11 wherein the ion chelator comprises a divalent ion chelator.

13. The method of claim 12 wherein the divalent ion chelator is selected from the group consisting of EDTA and EGTA.

14. The method of claim 10 wherein the step of exposing the sample to a minimum amount of an inhibitor of DNA degradation comprises providing EDTA in a range from 0.042 g EDTA per gram of stool to 0.782 g EDTA per gram of stool.

15. The method of claim 9 further comprising extracting a target DNA.

16. The method of claim 9 wherein the step of extracting comprises a phenol-chloroform extraction.

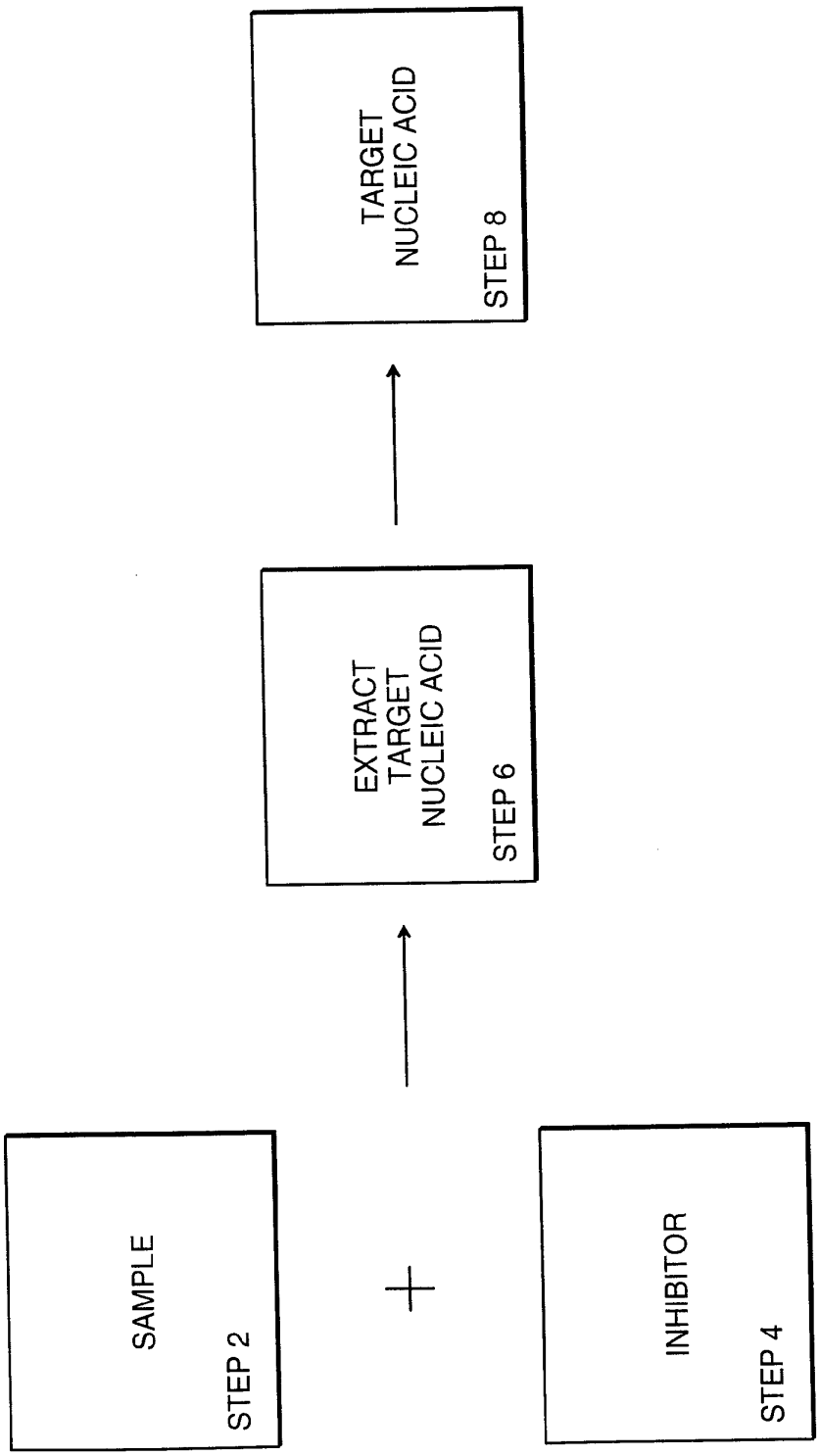

TARGET NUCLEIC ACID
STEP 8
EXTRACT TARGET NUCLEIC ACID
STEP 6
SAMPLE
STEP 2
INHIBITOR
STEP 4

FIG. 1

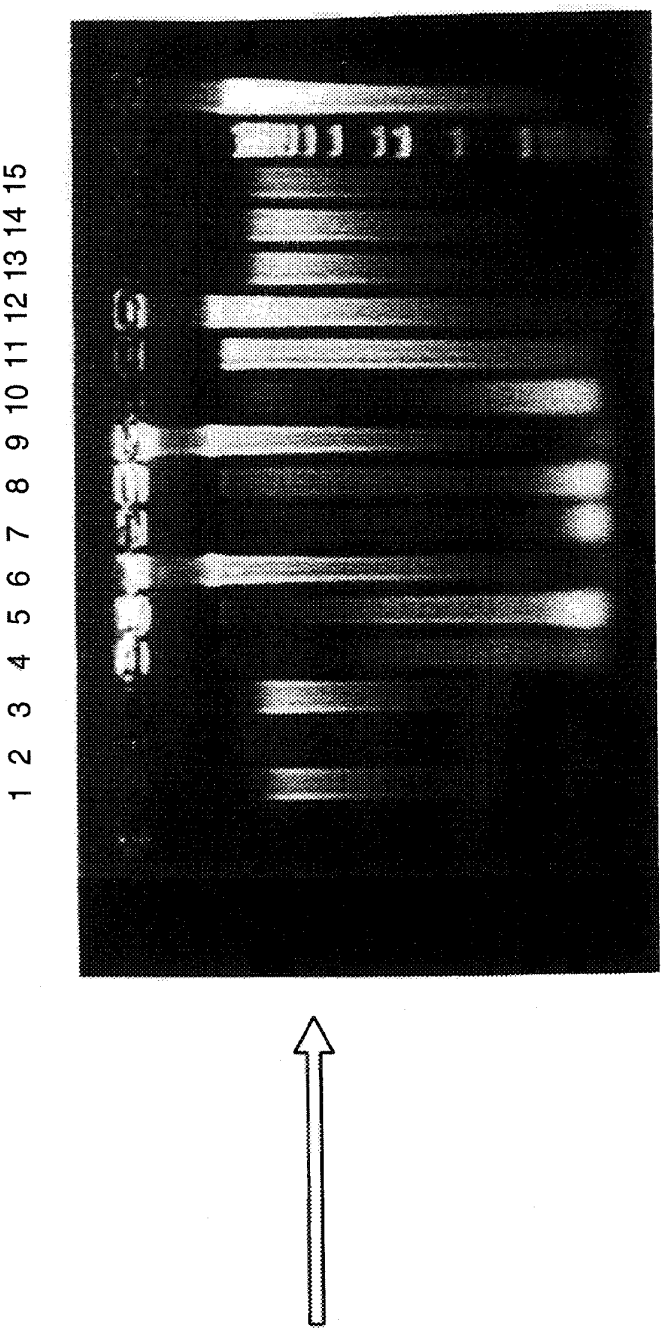


FIG. 2

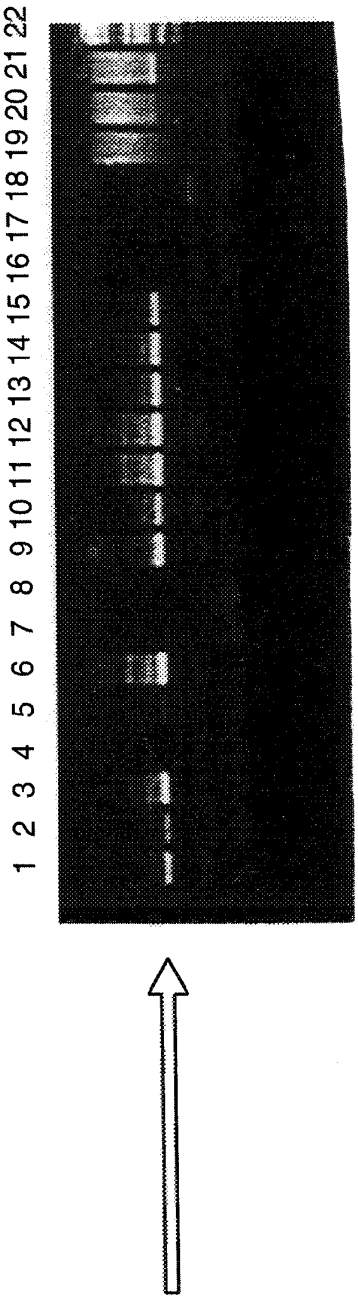


FIG. 3

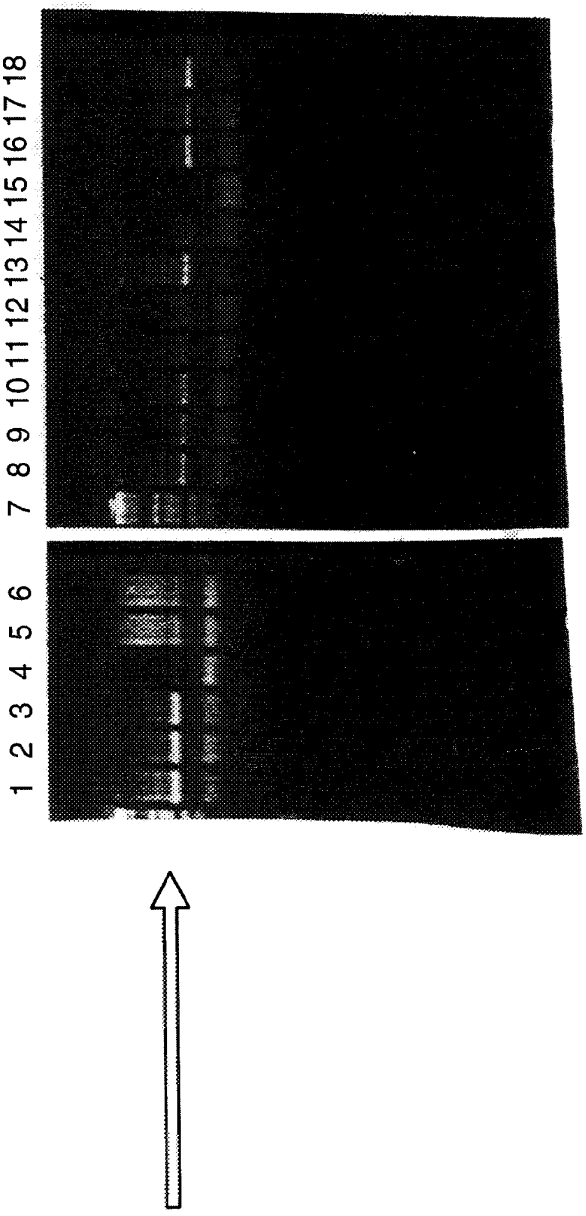


FIG. 4

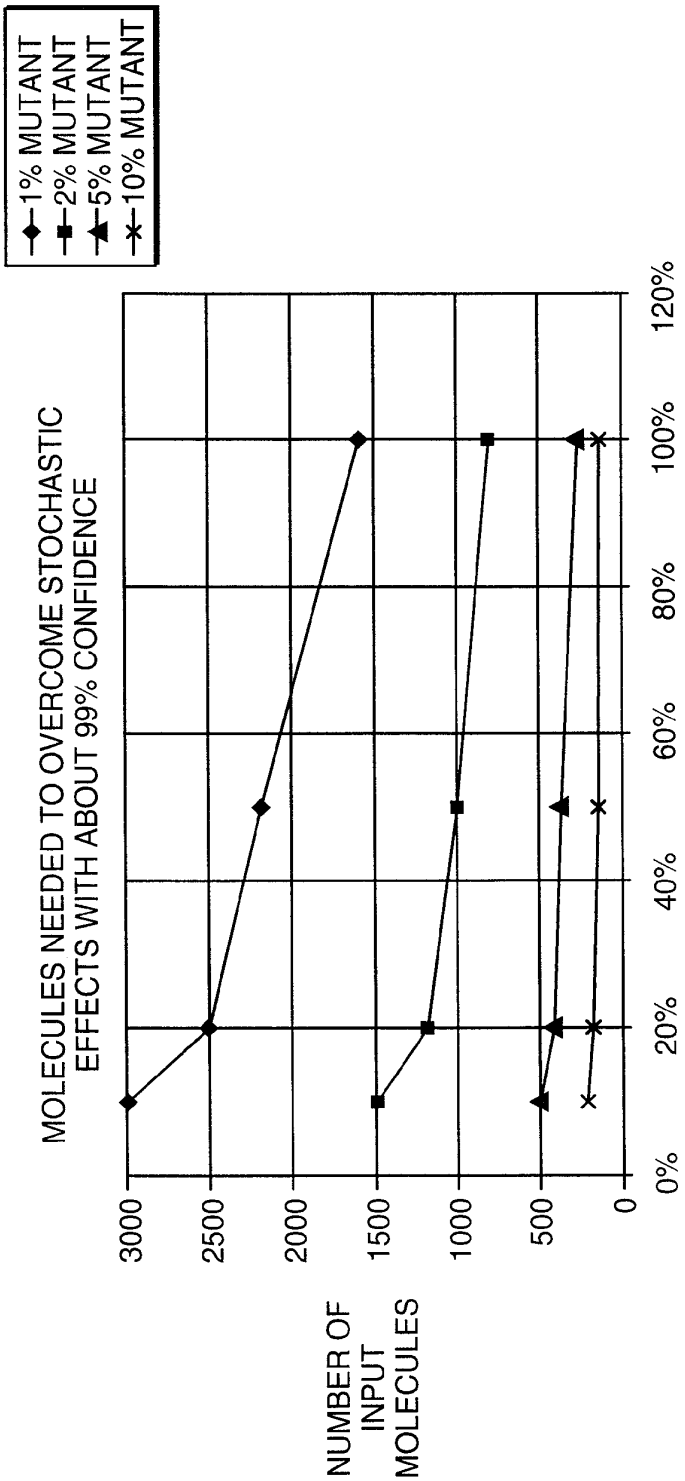

1% MUTANT
2% MUTANT
5% MUTANT
10% MUTANT
MOLECULES NEEDED TO OVERCOME STOCHASTIC EFFECTS WITH ABOUT 99% CONFIDENCE
NUMBER OF INPUT MOLECULES
3000
2500
2000
1500
1000
500
0
0%
20%
40%
60%
80%
100%
120%
PCR EFFICIENCY

FIG. 5

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 00/01759

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 20235 A (UNIV JOHNS HOPKINS MED) 14 October 1993 (1993-10-14) the whole document ---	1-16
X	WO 98 58081 A (EXACT LAB INC) 23 December 1998 (1998-12-23) the whole document ---	1-16
X	DE 195 30 132 A (MAX PLANCK GESELLSCHAFT) 20 February 1997 (1997-02-20) the whole document ---	1-16
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2000	04/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hagenmaier, S

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 00/01759

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CALDAS C ET AL: "DETECTION OF K-RAS MUTATIONS IN THE STOOL OF PATIENTS WITH PANCREATIC ADENOCARCINOMA AND PANCREATIC DUCTAL HYPERPLASIA" CANCER RESEARCH,US,AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 54, 1 July 1994 (1994-07-01), pages 3568-3573, XP002050203 ISSN: 0008-5472 the whole document ---	1-16
X	SIDRANSKY ET AL.: "IDENTIFICATION OF RAS ONCOGENE MUTATIONS IN THE STOOL OF PATIENTS WITH CURABLE COLORECTAL TUMORS" SCIENCE, vol. 256, 1992, pages 102-105, XP000605488 the whole document -----	1-16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int .tional Application No
PCT/US 00/01759

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9320235 A	14-10-1993	CA 2132874 A	14-10-1993
		EP 0672181 A	20-09-1995
		JP 8504081 T	07-05-1996
		US 5910407 A	08-06-1999
WO 9858081 A	23-12-1998	AU 7816898 A	04-01-1999
DE 19530132 A	20-02-1997	AU 712331 B	04-11-1999
		AU 6821696 A	12-03-1997
		CA 2228769 A	27-02-1997
		WO 9707239 A	27-02-1997
		EP 0851937 A	08-07-1998
		JP 11511020 T	28-09-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
				Filing Date	06/27/2017
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	1634
				Examiner Name	WHISENANT
Sheet	1	of	2	*Attorney Docket Number*	EXCTD-35239.304

U.S. PATENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		6761702	2004-07-13	SMITH	

U.S. PUBLISHED PATENT APPLICATIONS					
Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20020096469	2002-07-25	FAULKNER	
		20030086869	2003-05-08	STALLINGS	
		20040019298	2004-01-29	ZHOU	
		20050075543	2005-04-07	CALABRESE	
		20050244836	2005-11-03	TSANG	
		20070072214	2007-03-29	GARVIN	

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] *(if known)*	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		WO 2000/050640	2000-08-31	EXACT LABORATORIES, INC.		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]

Examiner Signature		Date Considered	

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
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Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
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PATENT CENTER #	61551831	AUTHORIZED BY	Mary Ann Brow
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1801	REQUEST FOR CONTINUED EXAMINATION (RCE) - 1ST REQUEST (SEE 37 CFR 1.114)	1360.00	1	1360.00
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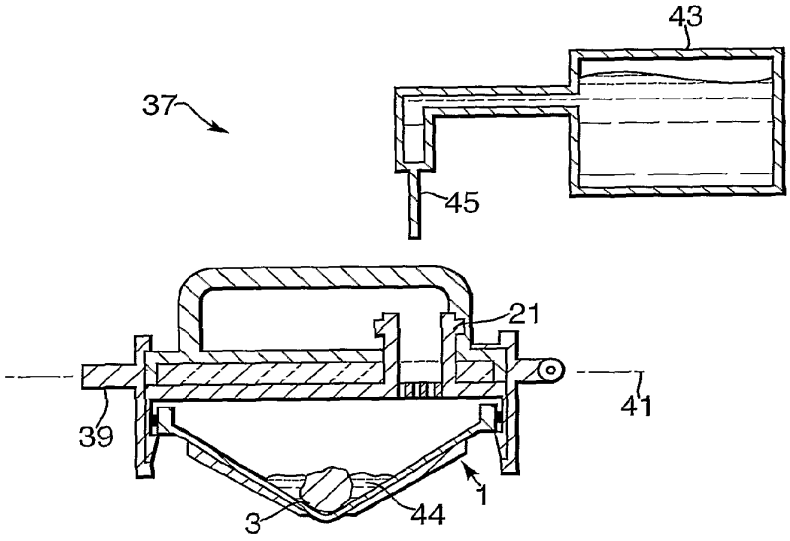
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(54) Title: METHOD AND APPARATUS FOR REMOVING CELLULAR MATERIAL FROM FAECAL STOOLS AND CONTAINER FOR COLLECTING FAECAL STOOLS



(57) Abstract: A method of removing cellular material from the surface of a faecal stool comprises the steps of: a) supporting the stool in a container having a channel along which the stool extends; b) cooling the stool to a temperature below its gel greezing point; c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool. Further disclosed are an apparatus (4) for removing cellular material from. the surface of a faecal stool and a container for collecting faecal stools (3b) .

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METHOD AND APPARATUS FOR REMOVING CELLULAR MATERIAL FROM FAECAL STOOLS AND CONTAINER FOR COLLECTING FAECAL STOOLS

Field of the Invention

The present invention relates to a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools.

Background

Faecal stools are the residue of digestive products in the gastrointestinal tract, and contain not only bacteria and undigested food residues, but also residual products from the body's metabolism and structure such as exfoliated epithelial cells from the lining of the gastrointestinal tract.

Exfoliated epithelial cells consist of cellular components, such as DNA, RNA and proteins, which potentially provide a multiplicity of approaches to identifying and monitoring unhealthy conditions of the epithelium as exemplified by colorectal cancer, polyps and ulcerative colitis. The facile recovery of such cells makes possible the application of a wide variety of test procedures for qualitative and/or quantitative analysis of, for example, DNA alterations, gene expression and enzyme activity. A. Loktionov *et al.*, in *Clinical Cancer Research*, Vol. 4, 337-342, (1998), describe a non-invasive screening test for early detection of colorectal cancer which is based on a quantitative comparison of the DNA concentrations derived from exfoliated epithelial cells isolated from the surface of stools from healthy and tumour-bearing persons. In this isolation procedure, cells on the surface of the stool were predominantly clustered and had varying degrees of intactness and damage. In the present specification by "cells" and "cellular material" we mean all such material irrespective of degree of clustering or intactness.

In our earlier patent application, WO 97/09600, we describe a method of isolating cells (especially exfoliated epithelial cells) from the surface of faecal stools, comprising cooling a stool to a temperature (preferably of -80°C to 15°C, preferably -10°C to 15°C, preferably -10°C to 10°C, more preferably 0°C to 5°C) below its gel freezing point and removing the cells from the cooled stool by agitating the stool in an aqueous solution (which may be pre-cooled to a temperature below the gel freezing point, and is preferably ice-cold). The cooled condition creates a much more physically robust hard gel state that helps to prevent the stool from breaking up or rapidly eroding so that non-surface material is largely prevented from entering the aqueous solution.

Stool surface layers provide a particularly rich source of exfoliated cells suitable for isolation. Typically between one-sixth and one third of colonic epithelial cells are exfoliated per day (D. Sidransky *et al.* (1992), *Science* 256, 102-105 and W. C. MacDonald, J. S. Trier, N. B. Everett, *Gastroenterology* (1964) 46, 405). It is believed that some of these exfoliated cells, together with mucus, may be envisaged as forming a "sheath" around a stool, containing a low proportion of bacterial cells and other faecal matter. It is further believed that the viability of the cells in the surface layers (i.e. the layer of faecal material within 1.0 mm, and preferably 0.25 mm, of the stool surface) of the stool is facilitated by i) a tendency of the cells to agglomerate into plaques (which helps to prevent apoptosis), ii) the availability of oxygen at the surface of the stool and iii) the unique ability of colonic epithelial cells to utilise as fuel the butyrate formed locally in high concentrations by microfloral fermentation (J. H. Cummings (1981) *Gut* 22, 763-799 and W. E. W. Roediger (1980) *Gut* 21, 793-798). Oxidation of butyrate is believed to provide fuel for the cells in the

absence of blood-borne nourishment following exfoliation. It has been found that isolation of cells from stool surface layers provides significant advantages in yield, purity and ease of recovery over isolation of cells from homogenised stools.

Summary of the Invention

Although the method described in WO 97/09600 is effective for isolating cells from the surfaces of faecal stools, in order to make the method more suitable for high throughput screening of e.g. hundreds or thousands of samples per day, problems remain of washing the stools in a standard and reproducible way that maximises the amount of surface cells extracted from the stools but minimises the risk of breaking up the stools.

Consequently, in a first aspect, the present invention provides a method of removing cellular material from the surface of a faecal stool comprising the steps of:

a) supporting the stool in a container having a channel along which the stool extends;

b) cooling the stool to a temperature below its gel freezing point;

c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and

d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool. A minority of stools are very soft when excreted, such that on cooling they do not reach the gel freezing point, but do become sufficiently viscous to be washed with minimal entry into the wash liquid of sub-surface material. Thus, for that minority of stools, by "gel freezing point" we mean the temperature at which the stools become sufficiently viscous to be washed with negligible entry of sub-surface material into the wash liquid.

The method promotes the reproducible and gentle washing of the stool, which facilitates the quantitative comparison of cellular material between individuals.

Human faecal stools are typically elongate and approximately cylindrical in shape. So the channel significantly reduces the likelihood that sections of the stool will break off when the stool is being washed because the channel walls substantially prevent side-to-side rolling motion of the stool and, for a wide range of rocking angles and speeds, friction largely prevents the stool from sliding along the channel. Preferably the container is moved to cause a wash frequency of from 5 to 40 back and forth cycles per minute, and more preferably from 15 to 25 cycles per minute. The stool may be pre-soaked (for e.g. 3 minutes) in the wash liquid prior to step d), in which case preferably the container is moved for a total time of from 10 seconds to 1 minute. Without a pre-soak, preferably the container is moved for a total time of from 30 seconds to 5 minutes. With or without pre-soaking it is advantageous to limit the exposure of the stool to the wash liquid so as to avoid localised depletion of wash reagents or over-enrichment of released cellular material. The channel may have a V or U-shaped transverse section.

An advantage of the container motion, is that the wash liquid is channelled back and forth along the length of the stool so that, compared with e.g. circular agitation by an orbital shaker of the wash liquid relative to the stool (which we have found in any event tends to break up the stool), less liquid is required to wash the cells from the surface of the stool. Clearly the less liquid that is used to wash the stool, the higher the resulting concentration of cells in the liquid, which can in turn improve the accuracy of screening tests which may be subsequently performed on the liquid. Also smaller volumes of liquid

reduce cost and disposal problems. Preferably from 30 to 150 ml of liquid is used in steps c) and d). This is generally sufficient to wash a typical 100 g stool while minimising losses due to residual wetting of the container and stool. Most preferably a fixed ratio of liquid to stool is used so that all stools experience a similar degree of wetting. This ratio may be in the range 0.5 to 1.0 ml of liquid per gram of stool.

In one embodiment, step d) comprises rocking the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity. In another embodiment, step d) comprises displacing the container back and forth in the direction in which the channel extends.

Preferably the container is of fixed shape, e.g. by being formed of rigid material.

In a second aspect, the present invention provides an apparatus for removing cellular material from the surface of a faecal stool, comprising:

mounting means for mounting a container having a channel along which, in use, the stool extends; and

motion imparting means for moving the container so that, when wash liquid is introduced into the container, the liquid runs back and forth along the channel thereby washing cellular material from the surface of the stool.

In one embodiment, the motion imparting means rocks the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity. In another embodiment, the motion imparting means displaces the container back and forth in the direction in which the channel extends.

The apparatus may further comprise control means to control e.g. the rocking angle, rocking or displacement frequency and/or total time of movement of the container, preferred ranges for some of these parameters being

provided above in respect of the first aspect of the invention. The control means may further control a sequence of stationary and mobile periods for the container, so that e.g. stationary exposure of the stool to the wash liquid allows the liquid to penetrate the stool and loosen its surface layers prior to active washing when the container is moved.

With such an apparatus, stool washing can be performed in a repeatable, standardised manner, and with a relatively high stool sample throughput. These are significant advantages if the wash liquid is to provide the material for a screening test based on e.g. a quantitative comparison of amounts of epithelial cell components.

The apparatus may further comprise cooling means for cooling the stool to a temperature (preferably of -80°C to 15°C, preferably -10°C to 15°C, preferably -10°C to 10°C, more preferably 0°C to 5°C) below its gel freezing point.

In one embodiment the apparatus further comprises a filter means such as a sieve, so that, when wash liquid is removed from the container, the cells are retained in the liquid but food residues from the stool are filtered from the liquid.

The apparatus may further comprise a metering device for introducing a metered dose (e.g. 50 to 150 ml, or an amount which is related to the stool weight) of wash liquid into the container, and/or a wash liquid pre-cooler for cooling the wash liquid to a temperature below the gel point of the stool before the wash liquid is introduced into the container. This temperature is preferably about 0°C.

The apparatus may further comprise at least one and preferably a plurality of detachable sample receptacles into which wash liquid is transferable from the container, so that e.g. when stool washing is completed the wash liquid may be poured from the container into the

receptacles. These can then be detached from the apparatus and transferred to an automated liquid-handling system for high throughput processing of the liquid samples contained therein.

A third aspect of the invention provides a fixed shape container for collecting faecal stools which has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel. The mouth can be of any suitable shape, e.g. rectangular or circular. The length of the channel is preferably in the range from 10 to 25cm, which corresponds to the size of a typical stool. The container may be used in the first or second aspect of the invention.

As faecal stool is generally regarded as disgusting material that people are reluctant to handle, even indirectly, the fixed shape container has several advantages as compared with, e.g. a flexible plastic specimen bag for collecting stool. For example, the mouth of the container cannot collapse as a stool is being deposited in the container, thereby making it easier for a user to excrete into the container. Also the container can be handled without, apart from the weight of the stool, any sensation that a stool is contained therein.

Furthermore the funnelling effect of the narrowing container walls guides a formed stool (which on defecation can be quite malleable) to a position in which it extends along the channel. This reduces or eliminates the need for the user to directly or indirectly manipulate the stool into that position prior to stool washing. Soft stools, and even diarrhoea, are also encouraged to align along the channel by the narrowing of the container walls.

A further advantage of the container is that it can serve both as the receptacle into which the stool is

excreted and as the container in which the stool is washed. So the stool does not need to be transferred to a different washing container, which avoids damage to the stool surface caused by such a transfer. Such damage would interfere with the accuracy of e.g. screening tests which may be performed on the stool.

If the stool is excreted into the container at home and then transported to e.g. a clinic for washing, the container has the further advantage of holding the stool reasonably securely in the channel so that transportation damage to the stool surface is reduced or avoided.

To avoid user embarrassment during transportation at least a majority of the container may be opaque (i.e. a stool inside the container is not visible through the opaque portions of the container, even if the material of the container transmits light to some degree), or an opaque transport casing may be provided for transporting the container. However, desirably at least a portion of the container permits viewing to the inside of the container so that e.g. after delivery of the container for stool processing a health professional can check the stool for gross abnormalities such as blood before the stool is processed and without opening the container.

The container may further have installation means (which may be detachable from the container) for removably installing the container on a toilet (e.g. below the toilet seat) with the receiving mouth opening upwardly and the collection channel extending horizontally beneath the mouth. This allows the user to produce the stool in the usual manner, which promotes user acceptability.

Typically, for hygiene reasons, at least the stool-contacting parts of the container are disposable. However, in one embodiment the internal walls of the container serve as a former for a bag which is reversibly fixable inside the container, whereby the bag assumes the shape of the

mouth and channel. In this embodiment the bag is typically disposed after use, but the container can be reused.

Preferably the container is made of plastics material such as polypropylene. Desirably the container has smooth internal surfaces which reduce damage to the surface of the stool as the stool passes from the mouth to the channel.

In one embodiment the container has cooling means, such as an outer jacket or bath, for cooling a stool deposited in the container. This means that the stool can be cooled immediately it is produced, which helps to preserve the surface layer cells and makes the stool robust for physical transport. The cooling means may be of material that can be pre-cooled in a domestic refrigerator or freezer. This is particularly convenient when the stool is produced at home, allowing the donor to defecate at a convenient time and in a minimally stressful setting and thereby promoting user acceptability. The cooling means may enable the stool to be cooled to a temperature below its gel freezing point.

The cooling means may be detachable from the container so that the cooling means can be pre-cooled before fitting to the container. Also, if the container is disposable, the cooling means can still be reused.

Anaerobic bacteria in stools produce damaging radicals when exposed to atmospheric oxygen (C. F. Babbs (1990) *Free Radical Biology and Medicine*, 8, 191-200). Such oxygen also oxidises the mucus on the surface of stools and encourages the growth of aerobic bacteria. So preferably the container is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein. The seal may be achieved by means of a lid or cover (e.g. having a snap-fit or screw fitting) for the mouth. A sealed container rapidly becomes anaerobic due to the high concentration of anaerobic bacteria in the stool, and this apparently largely prevents the growth of aerobic

bacteria on the stool surface, helping to preserve the stool. We have found that sealed containers allow stools to be stored at 0°C for a number of weeks apparently without significant degradation. To promote user acceptability, the seal should also eliminate any odour from the stool escaping from the container, particularly if the stool is produced at home and then transported before washing.

The container may also have a liquid port which allows wash liquid to be removed from the container while maintaining the stool in the container. For example the diameter of the port may be sized to prevent a typical-sized stool from exiting via the port, or a foraminous screen over the port may serve the same function. The port may include closure means such as a valve or a cap which allows the liquid to be poured or sucked out of the container but at other times maintains an airtight seal. The same or another port may be used to introduce wash liquid into the container.

A further aspect of the invention provides a kit of parts comprising the container of the previous aspect with the installation means and/or the cooling means and/or the transport casing.

Another aspect of the invention provides a method of screening for unhealthy condition(s) of the epithelium (such as colorectal cancer, polyps and ulcerative colitis) in a plurality of subjects (which are typically human subjects, but may be other animal species such as dogs and cats), the method comprising the steps of:

a) providing to the subjects respective containers (e.g. according to the third aspect of the invention) for defecation of a stool sample thereinto at the convenience of the subject (which is typically when the subject is "at home" or in a domestic setting);

b) collecting the filled containers from the subjects at a central location (which may be a doctor's surgery or a hospital out-patients clinic);

c) removing a specimen of cellular material from the surface of each stool sample (e.g. according to the first aspect of the invention); and

d) testing each specimen for unhealthy condition(s) of the epithelium (e.g. as described by A. Loktionov *et al.*, referenced above).

Brief Description of the Drawings

The present invention is now described in respect of a specific embodiment and with reference to the accompanying drawings in which:

Figs. 1a and b show schematically and respectively a cross section through and a plan view of a receptacle for collecting a faecal stool, and Fig. 1c shows a plan view of an alternative form of the receptacle;

Fig. 2 shows the same cross section as Fig. 1a except that a lid is fixed to the receptacle;

Fig. 3a shows a schematic cross section of the receptacle of Fig. 1a fixed inside a transport unit, and Fig. 3b shows the receptacle of Fig. 1c fixed in another version of the transport unit;

Fig. 4 shows schematically a stool washing apparatus with the receptacle of Fig. 1a positioned in a sample rack; and

Fig. 5 shows the apparatus of Fig. 4 with the sample rack pivoted to pour wash liquid out of the receptacle.

Detailed Description

Figs. 1a and b show respectively a cross section through and a plan view of a transparent plastic receptacle 1 having a V-shaped cross-section for collecting a faecal stool 3 produced by a user. The receptacle has a mouth 5 and a straight stool collection channel 7 (which extends perpendicularly to the plane of the paper in Fig. 1a). The

receptacle is removably installed inside a toilet bowl 9 by a support web 11 which is attached over the rim of the toilet and has a central hole 12 to receive the receptacle so that channel 7 is horizontal and mouth 5 is directly above channel 7. The smoothly sloping walls 14 of the receptacle guide the stool to the bottom of channel 7 and encourage the deposited stool to align with the longitudinal direction of the channel.

Fig. 1c shows an alternative form of the receptacle which has a U-shaped channel cross-section, and which is capable of receiving larger specimens.

Once the stool is collected in the receptacle, a lid 13 (see Fig. 2) is used to close mouth 5 and is releasably fixed to the receptacle by a snap-fit mechanism 16. A sealing ring 15 seals the receptacle from the external atmosphere.

Lid 13 includes a carrying handle 17, thermally insulated upper wall 19 and wash liquid entry/exit port 21. The port is sealed by closure cap 23 and has a foraminous screen 25 at its inner end which prevents the stool from entering the port when (as described below) wash liquid is decanted from the receptacle, and a coarse sieve 25a to retain particles of e.g. greater than 2 mm in diameter.

The receptacle is lifted by handle 17 from web 11 and fixed inside a transport unit 27 by a snap-fit mechanism 29 (see Fig. 3a). The transport unit has thermally insulated walls 31 and contains a cooling block 33 of pre-cooled ice or chemical cooling medium contained under a shaped divider 35. The cooling block is brought into close proximity with the outer wall of receptacle 1. Ring 15a maintains the position of channel relative to the cooling block. The cooling block cools stool 3 to a temperature preferably in the range from 0 to 5°C, which is below the gel freezing point of the stool.

Fig. 3b shows the receptacle of Fig. 1c fixed in another version of the transport unit. In this version divider 35 forms an air space around the receptacle. The air space and divider 35 prevent the cooling block cooling the stool so rapidly as may damage the outer surface layers of the stool.

The stool is then transported in the transport unit to e.g. a clinic where stool washing is performed. The walls of the transport unit and lid are opaque so that the stools are not visible inside the unit, and lid 13 prevents odours from escaping the unit. Channel 7 also reduces movement of the stool during transportation which prevents significant damage to the surface of the stool.

At the clinic, the receptacle 1 and lid 13 are removed from the transport unit. As the receptacle is transparent visual inspection of the stool is possible without removing the lid. At this stage the stool sample may be rejected if, for example, it is too small or runny. also any gross evidence of disease, such as blood, may be noted. However, if the stool is acceptable, the receptacle and lid are positioned into the sample rack 39 (i.e. receiving means) of a stool-washing apparatus 37 shown in Fig. 4. Collection channel 7 is aligned perpendicular to axis 41 about which rack 39 is pivotable and which at this stage is horizontal.

Cooling means (not shown), such as a cold air jet, refrigerator unit or coolant bath, keeps or brings the stool to a temperature in the range 0 to 5°C so that it is below its gel freezing point.

Apparatus 37 has a reservoir 43 of chilled wash liquid, such as a buffered aqueous solution containing N-acetylcysteine, bovine serum albumin and sodium butyrate which has been found (see WO 97/09600) to improve recovery of exfoliated cells. When the stool is sufficiently cool, closure cap 23 is removed and a metered dose of wash liquid

44 is injected into the receptacle via an injector 45 and port 21. The liquid may alternatively be injected through an air-tight septum at port 21. Afterwards injector 45 is swung away from the sample rack.

Rocking means (not shown), which is e.g. an electric motor and associated drive, then rocks the sample rack and receptacle backwards and forwards about axis 41. A control unit (also not shown) which controls the rocking means defines a wash programme consisting of rocking angle, rocking frequency and total exposure and rocking time experienced by the stool. As receptacle 1 moves, gravity causes wash liquid 44 in the receptacle to run back and forth along channel 7 so that intimate contact with the stool is achieved and surface cells from the stool are washed into the liquid. To a significant extent friction prevents the stool from moving during the rocking procedure.

When the wash programme ends, the sample rack is pivoted about pivot 47 to bring axis 41 into a vertical position (see Fig. 5). Almost all of wash liquid 44 is then decanted out of receptacle 1 via port 21, foraminous screen 25 substantially preventing larger food residues or other broken off pieces from the stool exiting with the liquid. Coarse sieve 25a removes smaller pieces from the liquid. Finer downstream sieves (not shown) further limit the maximum particle size in the liquid. The liquid is then be divided into standard volumes and received into a plurality of removable (e.g. clip-on) sample receptacles (not shown). The sample receptacles may be a strip of tubes each of e.g. 0.1-0.5 ml volume as can be removed and clipped into a standard microplate format used for clinical analyses. Alternatively the sample receptacles may be individual tubes each of e.g. 1-2 ml volume. The sample rack is then returned to its horizontal position and receptacle 1 is removed and safely disposed of.

In this way, the apparatus converts a typical total 100-500 g mass of stool and wash liquid into aliquots of cell-suspension of typically only 50-1000 mg (i.e. a thousand-fold reduction in scale). The aliquots can be made representative by preventing the cells from settling out during the decanting process the aliquots. One method of preventing settling out is to provide mixing channels (not shown) through which the liquid flows on its way to the sample receptacles. Alternatively a stirrer (also not shown) can be provided to stir the wash liquid. Fine sieves (e.g. of water wettable material such as nylon, and/or having a mesh size in the range 50-400 μm, preferably 100-250 μm, more preferably 125-200 μm) may be provided over the entrances to the receptacles to further filter the liquid. The excess liquid flows to waste.

The samples may be used for e.g. DNA assays as described in WO 97/09600 and A. Loktionov *et al.*, *Clinical Cancer Research*, Vol. 4, 337-342, (1998).

The entire procedure, from when the receptacle is positioned in the sample rack to when the wash liquid exits the receptacle, can be automated, with standard wash programme settings being used. Also no manual contact with or manipulation of the stool required, which promotes good hygiene. So the present invention allows a large number of stool samples to be processed in a safe and reproducible manner.

In an experimental study, stools were collected into re-sealable bags and stored at 0°C from no more than a few minutes after excretion. The bags were transparent, which allowed visual inspection of the stools during testing. For stool washing, a wash solution was poured into each bag and the bag was located inside a rigid rocking channel of a stool washer apparatus, the channel being 40-45mm wide and perforated so as to permit intimate cooling by surrounding

ice-water held in an insulated container equipped with a submersible pump. Rocking caused a gravity-imposed back and forth circulation of the wash solution along the channel and also promoted cooling of the stool by the surrounding ice-water. A frequency of 3 seconds per rocking cycle was chosen.

The wash solution was as previously described by A. Loktionov *et al.* (referenced above), but the stool wash was passed through a 250 µm instead of a 125 µm sieve. Cell clean-up was performed robotically on a TECAN™ Genesis RSP 150 and then DNA was released by mechanical cell disruption in the presence of 0.9%SDS as lysing agent. Following dilution to prevent quenching, fluorimetric DNA assay was performed with a TECAN™ Spectrafluor Plus using PicoGreen (Molecular Probes™) to yield the stool DNA index (SDNAI, defined as nanograms of DNA per ml of stool wash).

The effects of pre-soaking, rocking angle, mechanical and bacterial damage, stool age, and wash volume were studied, and the results are given below.

Effect of pre-soaking on SDNAI

The following SDNAI data from the stool washer (using a +/-45deg rocking angle) demonstrate the advantage of a period of pre-soaking. Presoaking appears to promote a more or less equilibrated suspension state of cellular material in the wash solution, which thereby helps to prevent the chance dynamic release of cellular material.

Rocking time	SDNAI (ng/ml) with no pre-soak			SDNAI (ng/ml) with 3 min pre-soak		
	stool 1	stool 2	stool 3	stool 4	stool 5	stool 6
30-40 sec	34	12	29	115	206	217
1 min	118	43	21	101	221	294
3 min	100	80	75	125	216	338

Effect of Rocking Angle

If too small a rocking angle was used or the channel became obstructed by the stool, the wash liquid tended to form non-flowing pools that failed to contact the stool surface so that unacceptable variations in washing condition were experienced. However, under favourable conditions, reproducible and relatively constant results could be obtained over a range of rocking angles. For example in three different experiments the same stool was given a 3 minute pre-soak followed by a 1 minute of wash at different rocking angles in a visibly free flow of liquid in a channel 40-45 mm wide and using a wash liquid to stool ratio of 0.7 ml per gram. The rocking angles for the three experiments were +/-30, 45 and 60° from the horizontal and the respective SDNAI measurements were 263, 254 and 260 ng/ml.

Effects of Damage to Stool Surface Layer and of Stool Age

Sometimes stored stools were found to be accidentally squashed, or stored in improperly sealed containers as evidenced by smalls amount of white bacterial over-growth on the stools. The following data (using different stools all washed for 1 minute after a 3 min pre-soak at a wash solution/stool weight ratio of 0.7) demonstrate the importance of protecting the stool surface layer during storage. However, they also demonstrate that under favourable conditions storage durations of up to several weeks have no particular effect on measured SDNAI values. The squashed stools had SDNAI values significantly above the 700 ng/ml value which is considered to be the upper limit for healthy persons.

Stool age (days) donor A	1	4	7	11[s]	15	19	81[s]
SDNAI	203	192	90	1,400	218	585	987

Stool age (days) donor B	14	29[b]	29[b]	31	42[b]	73	101
SDNAI	255	23	50	177	78	261	515

[s] indicates the stool was squashed

[b] indicates the stool had bacterial over-growth

Effect of Wash Solution Volume

Each of a series of undamaged stools from one donor was washed (with a 3 min pre-soak and a 3 min wash at +/- 45° rocking angles) with both a fixed 50 ml volume of wash solution and a variable volume of wash solution based on a 0.5 ml/gram wash/stool ratio. For successive stools of the series, washing first was alternated between the fixed and variable volume. The variable volume based on the 0.5 ratio resulted on average in the use of a 64% greater volume of wash solution which visibly appeared to wet the whole stool better and produced a 21% greater DNA concentration. The second washes gave a 54% higher on average DNA yield than the first washes (largely irrespective of whether the fixed or variable wash solution volume was used first) which was presumably due to the pre-softening effect of the first washes. In general therefore the fixed ratio wash took better account of the stool weight without significantly influencing the DNA result. As shown above, stool age had an insignificant effect on the results.

stool age (days)	1	5	9	20	60	70
stool weight (g)	89	141	103	232	274	146
SDNAI from 50 ml wash	1st 55	2nd 167	1st 234	2nd 300	2nd 256	1st 49
SDNAI from 0.5 ratio wash	2nd 139	1st 92	2nd 220	1st 262	1st 207	2nd 65

Claims:

1. A method of removing cellular material from the surface of a faecal stool comprising the steps of:

a) supporting the stool in a container having a channel along which the stool extends;

b) cooling the stool to a temperature below its gel freezing point;

c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and

d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool.

2. A method of removing cellular material according to claim 1, wherein step d) comprises rocking the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity.

3. A method of removing cellular material according to claim 1, wherein step d) comprises displacing the container back and forth in the direction in which the channel extends.

4. A method of removing cellular material according to any one of claims 1 to 3, wherein the container has a fixed shape.

5. An apparatus for removing cellular material from the surface of a faecal stool, comprising:

mounting means for mounting a container having a channel along which, in use, the stool extends; and

motion imparting means for moving the container so that, when wash liquid is introduced into the container,

the liquid runs back and forth along the channel thereby washing cellular material from the surface of the stool.

6. An apparatus for removing cellular material according to claim 5, wherein the motion imparting means rocks the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity.

7. An apparatus for removing cellular material according to claim 5, wherein the motion imparting means displaces the container back and forth in the direction in which the channel extends.

8. An apparatus for removing cellular material according to any one of claims 5 to 7, further comprising cooling means for cooling the stool to a temperature below its gel freezing point.

9. An apparatus for removing cellular material according to any one of claims 5 to 8, further comprising filter means which filters food residues from the stool but retains the cellular material in the wash liquid when the liquid is removed from the container.

10. An apparatus for removing cellular material according to any one of claims 5 to 9, in which the container is mounted at the mounting means.

11. A container for collecting faecal stools which has a fixed shape and has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel.

12. A container according to claim 12, further having installation means for removably installing the container on a toilet with the receiving mouth opening upwardly and the collection channel extending horizontally beneath the mouth.

13. A container according to claim 11 or 12, further having cooling means for cooling a stool deposited in the container.

14. A container according to any one of claims 11 to 13, which is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein.

Fig.1a.

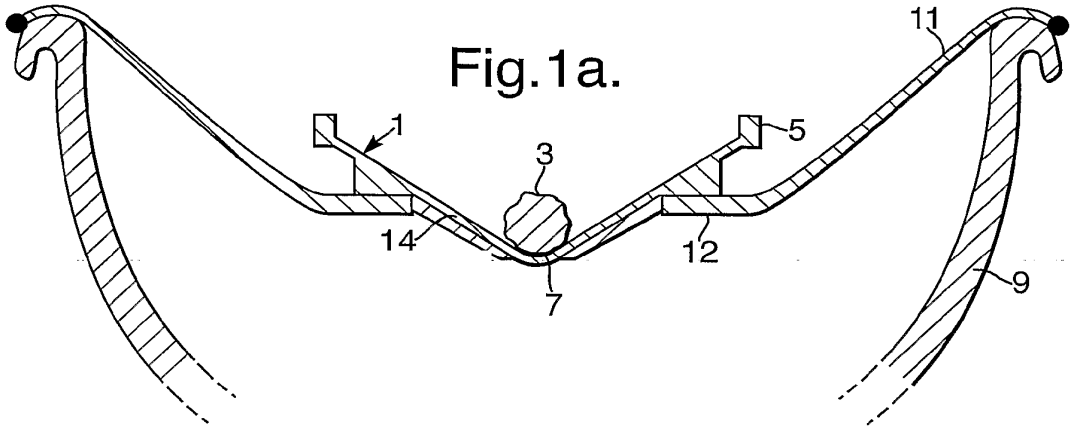


Fig.1b.

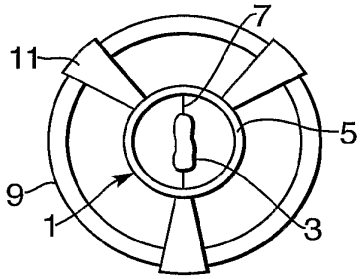


Fig.2.

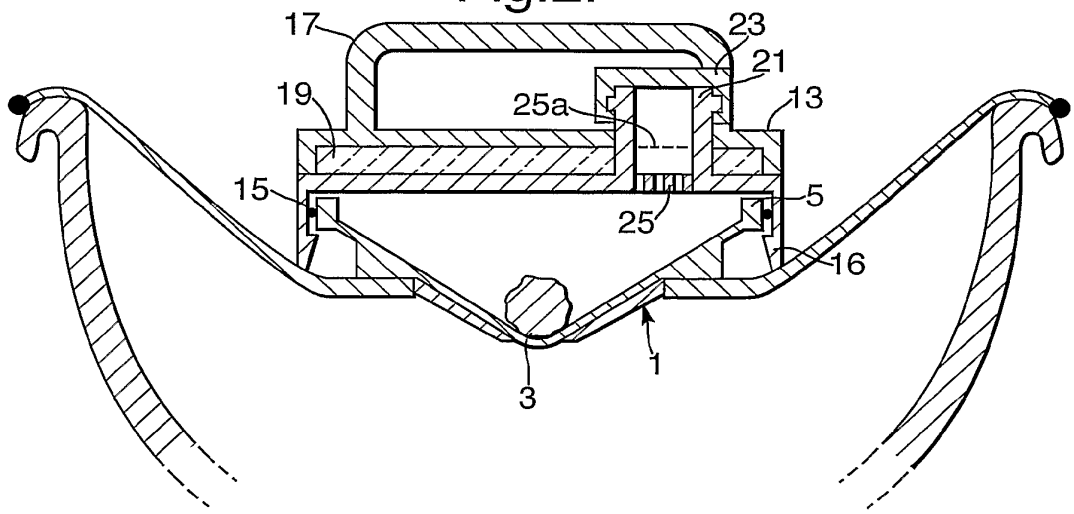


Fig.1c.

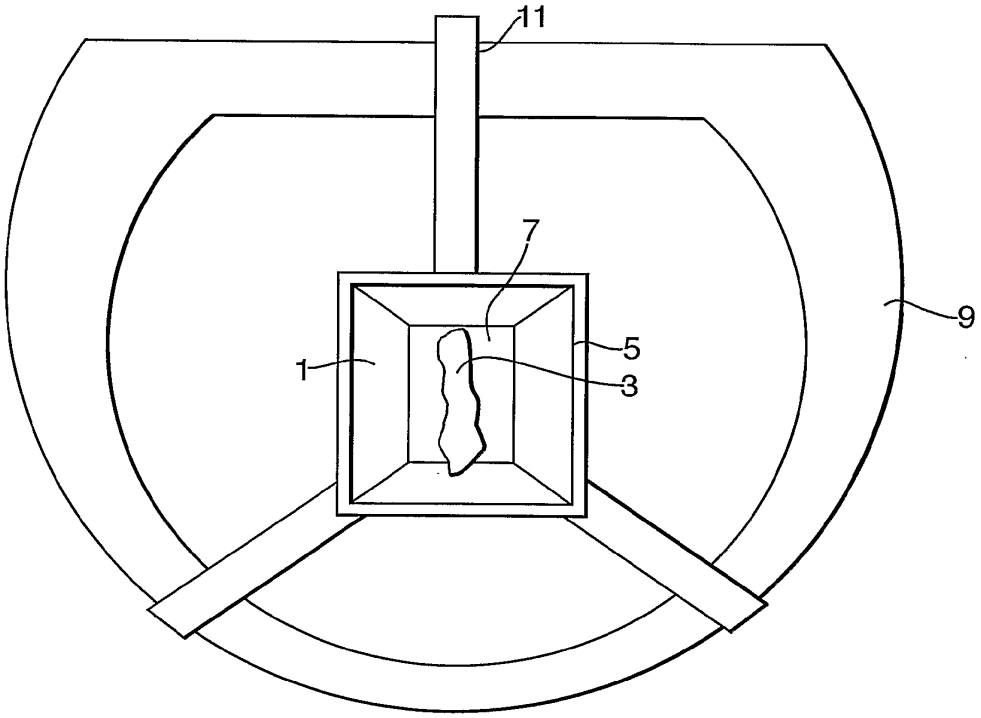


Fig.3a.

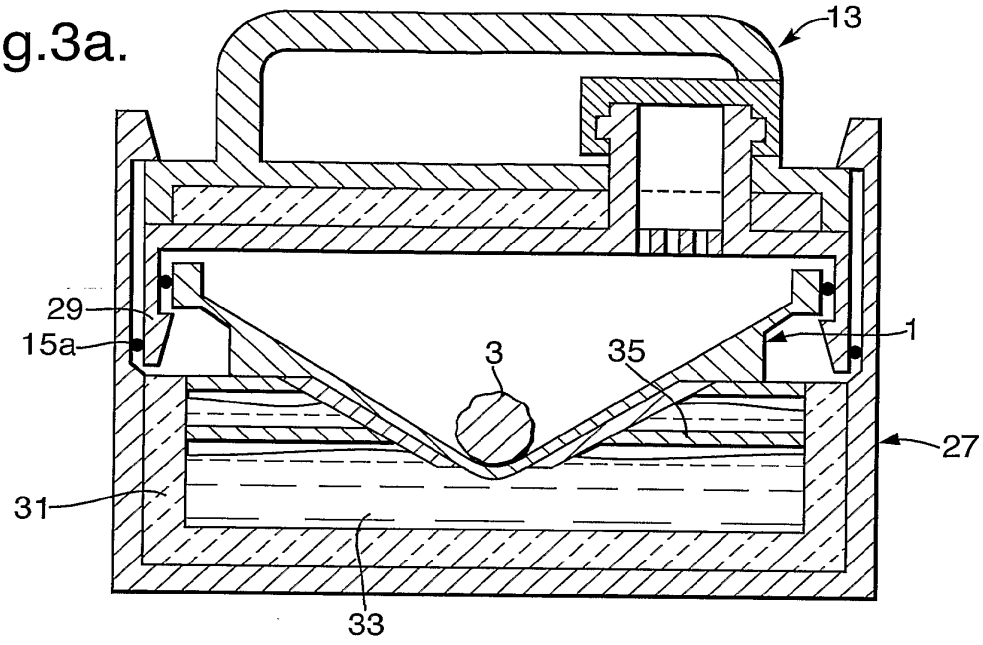

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Fig.3b.

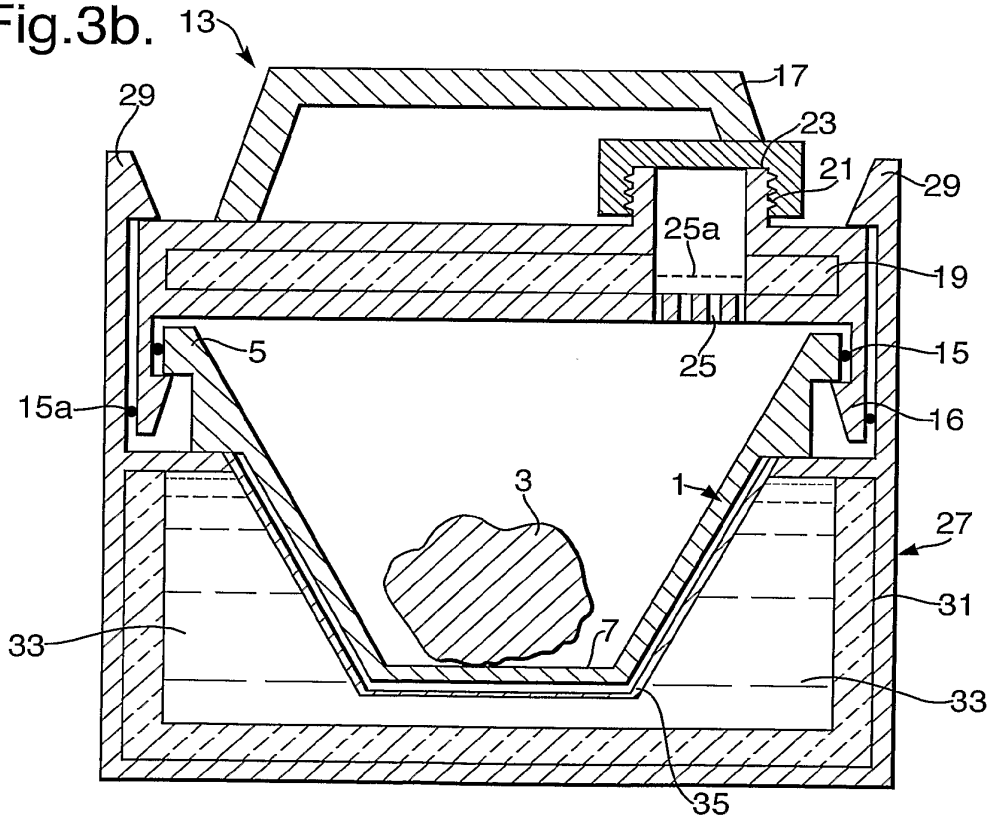

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Fig.5.

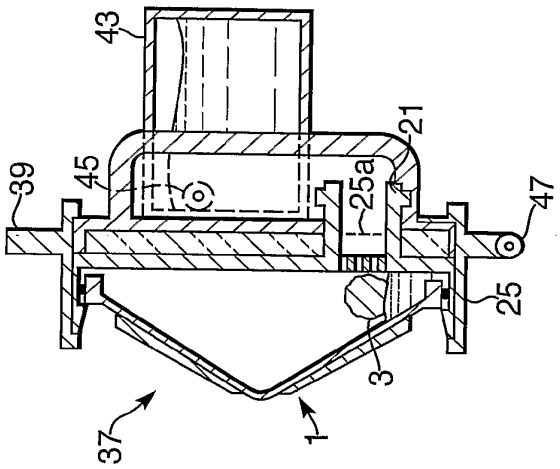
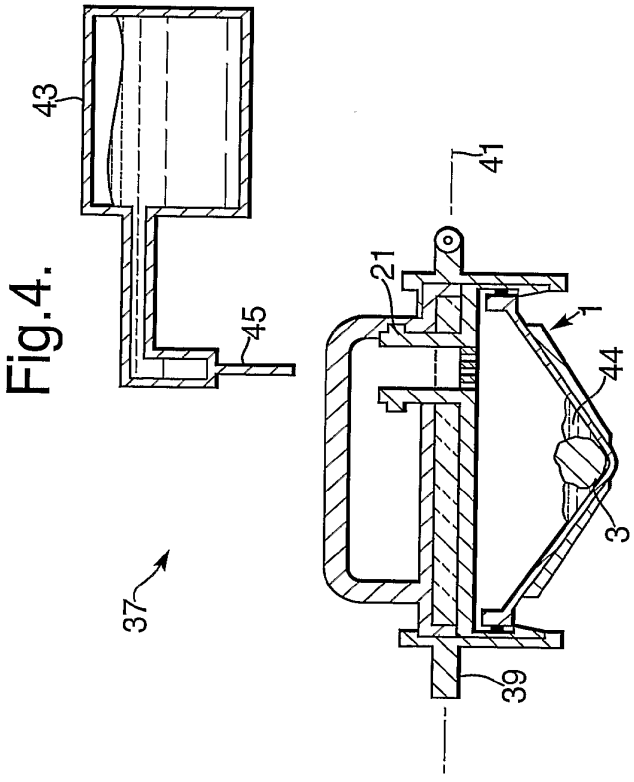


Fig.4.



INTERNATIONAL SEARCH REPORT

International Application No
B2004/003434

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01F11/00 C12Q1/68 A61B10/00 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01F C12Q A61B G01N A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 187 546 B1 (LOKTIONOV ALEXANDRE ET AL) 13 February 2001 (2001-02-13) cited in the application column 4, line 47 - column 5, line 9 column 13	1-4
X	US 2001/002985 A1 (KLEINSASSER JONATHAN) 7 June 2001 (2001-06-07) paragraphs '0052! - '0058!; figures 6,7	5,6,8,10
X	DE 15 98 515 A (GERHARDT FABRIK UND LAGER CHEM) 27 May 1971 (1971-05-27) the whole document	5,7,10
A	US 2002/119472 A1 (LAPIDUS STANLEY N ET AL) 29 August 2002 (2002-08-29) paragraph '0038!	1-4

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2004	11/11/2004

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Int[illegible]al Application No
PCT/GB2004/003434

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 456 507 A (BRACONNIER THERESE) 12 December 1980 (1980-12-12)	11,14
Y	figure 1	13
Y	DE 199 10 836 A (KERNFORSCHUNGSANLAGE JUELICH) 21 September 2000 (2000-09-21) abstract	13

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ınal Application No
[illegible] B2004/003434

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 6187546	B1	13-02-2001	AU	6883096 A	27-03-1997
			EP	0873502 A2	28-10-1998
			WO	9709600 A2	13-03-1997
			JP	11511982 T	19-10-1999
US 2001002985	A1	07-06-2001	AU	1574102 A	18-06-2002
			WO	0245619 A2	13-06-2002
			CA	2430758 A1	13-06-2002
DE 1598515	A	27-05-1971	DE	1598515 A1	27-05-1971
US 2002119472	A1	29-08-2002	US	6303304 B1	16-10-2001
			US	5952178 A	14-09-1999
			US	5741650 A	21-04-1998
			AU	3491999 A	01-11-1999
			CA	2328616 A1	21-10-1999
			EP	1073898 A1	07-02-2001
			JP	2002511585 T	16-04-2002
			WO	9953316 A1	21-10-1999
			AT	246806 T	15-08-2003
			AU	704696 B2	29-04-1999
			AU	2240697 A	22-08-1997
			CA	2215263 A1	07-08-1997
			DE	69629363 D1	11-09-2003
			DE	69629363 T2	09-06-2004
			DK	817968 T3	01-12-2003
			EP	1291657 A2	12-03-2003
			EP	0817968 A1	14-01-1998
			ES	2202497 T3	01-04-2004
			JP	2002515973 T	28-05-2002
			WO	9728450 A1	07-08-1997
FR 2456507	A	12-12-1980	FR	2456507 A1	12-12-1980
DE 19910836	A	21-09-2000	DE	19910836 A1	21-09-2000

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
15/634,607	**02/06/2023 03:43:08 PM ET**	**EXCTD-35239.304**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1874	FILED BY	Stephanie Filandrinos
PATENT CENTER #	61551831	FILING DATE	06/27/2017
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 5

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-02-06_RCE-Form_EXCTD-003USCON2-35239_35239-304.pdf	3	Request for Continued Examination (RCE)	1628 KB
2023-02-06_IDS-SB08_EXCTD-003USCON2.35239_35239.pdf Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.	3	Information Disclosure Statement (IDS) Form (SB08)	162 KB
sb0439.pdf	2	Internet Communications Authorization	190 KB

WO2000050640A1.pdf	33	Foreign Reference	1298 KB
WO2005014154A1.pdf	31	Foreign Reference	1267 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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New International Application Filed with the USPTO as a Receiving Office

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Doc Code: ECOMM.AUTH/ECOMM.WTDW
Doc Description: Internet Communications Authorization/Internet Communications Authorization Withdrawal

PTO/SB/439 (11-15)

AUTHORIZATION FOR INTERNET COMMUNICATIONS IN A PATENT APPLICATION OR REQUEST TO WITHDRAW AUTHORIZATION FOR INTERNET COMMUNICATIONS		
	Application No.	15/634,607
	Filing Date	06/27/2017
	First Named Inventor	JOOST LOUWAGIE
	Art Unit	1634
	Examiner Name	ETHAN WHISENANT
	Practitioner Docket No.	EXCT-34706.303

To: Commissioner for Patents
P.O. Box 1450
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II. To withdraw authorization for Internet Communications.

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I am the

☐ applicant.

☒ attorney or agent of record. Registration number 42363.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Mary Ann D. Brow/ — Signature

02/06/2023 — Date

Mary Ann D. Brow — Typed or printed name

608-662-1277 — Telephone Number

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

173254 7590 03/29/2023

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
WHISENANT, ETHAN C

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 03/29/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	06/29/2023

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

173254 7590 03/29/2023
CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	06/29/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
WHISENANT, ETHAN C	1634	435-006110

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via Patent Center or EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/634,607	06/27/2017	Joost Louwagie	EXCTD-35239.304	1874

173254 7590 03/29/2023

CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
WHISENANT, ETHAN C	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 03/29/2023

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/634,607	Applicant(s) Louwagie, Joost	
	Examiner Ethan C Whisenant	Art Unit 1634	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 06 FEB 2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 82,84,87,89-94 and 96-100 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 06 FEB 2023.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634

NOTICE OF ALLOWABILITY

► A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed on 06 FEB 2023 in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Claims 82, 84, 87, 89-94 and 96-100 are pending.

► The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

REASON(S) FOR ALLOWANCE

► **Claim(s) 82, 84, 87, 89-94 and 96-100** is/are deemed to be allowable for the reason(s) of record , see the NOA mailed 02 DEC 2022. The Claims have now been renumbered as Claims 1-14 in the same order as presented by the applicant.

CONCLUSION

C. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen, can be reached at (571) 272-0731.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS										
☑ Claims renumbered in the same order as presented by applicant			☐ CPA		☐ T.D.		☐ R.1.47			
CLAIM		**DATE**								
Final	Original	03/25/2023								
	1	-								
	2	-								
	3	-								
	4	-								
	5	-								
	6	-								
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	37	-								
	38	-								
	39	-								
	40	-								
	41	-								
	42	-								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/25/2023								
	43	-								
	44	-								
	45	-								
	46	-								
	47	-								
	48	-								
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2	84	=								
	85	-								
	86	-								
3	87	=								
	88	-								
4	89	=								
5	90	=								
6	91	=								
7	92	=								
8	93	=								
9	94	=								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CLAIM		DATE								
Final	Original	03/25/2023								
	95	-								
10	96	=								
11	97	=								
12	98	=								
13	99	=								
14	100	=								

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Ethan C Whisenant	1634

CPC - Searched*		
Symbol	**Date**	**Examiner**
C12Q 16886	11/22/2022	ECW
G01N 33/574	11/22/2022	ECW
Updated Search	03/24/2023	ECW

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	Art Unit
	Ethan C Whisenant	1634

Search Notes		
Search Notes	**Date**	**Examiner**
EAST (see transcript for databases and search terms)	06/23/2019	B. L. S.
Reviewed 15/010436 & 13/147570	06/23/2019	B. L. S.
EAST (see transcript for databases and search terms)	01/14, 15/2020	B. L. S.
STN (see transcript for databases and search terms)	01/14/2020	B. L. S.
EAST (updated)	08/04/2020	B. L. S.
EAST	06/16/2021	B. L. S.
EAST	03/22/2022	B. L. S.
PE2E Search	06/09/2022	B. L. S.
See the Examiner's Search Notes on the last pg of the NOA	11/22/2022	ECW
Updated Search	03/24/2023	ECW

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
C12Q	1/6886	11/22/2022	ECW
G01N	33/574	11/22/2022	ECW
Updated	Search	03/24/2023	ECW

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CPC						
Symbol					**Type**	**Version**
C12Q	/	1	/	6886	F	2013-01-01
G01N	/	33	/	57446	I	2013-01-01
C12Q	/	2600	/	106	A	2013-01-01
C12Q	/	2600	/	154	A	2013-01-01
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G01N	/	2800	/	50	A	2013-01-01
G01N	/	2800	/	52	A	2013-01-01
G01N	/	2333	/	805	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
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NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	24 March 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office — Part of Paper No.: 20230325

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/634,607	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

INTERNATIONAL CLASSIFICATION				
CLAIMED				
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G01N33/574	/	33	/	574

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US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

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CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
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(Primary Examiner)	(Date)	1	None

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	Ethan C Whisenant	1634

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

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	12		25		38		51		64		77	5	90		
	13		26		39		52		65		78	6	91		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
				Filing Date	06/27/2017
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	1634
				Examiner Name	WHISENANT
Sheet	1	of	2	Attorney Docket Number	EXCTD-35239.304

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		6761702	2004-07-13	SMITH	

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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		20030086869	2003-05-08	STALLINGS	
		20040019298	2004-01-29	ZHOU	
		20050075543	2005-04-07	CALABRESE	
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] (if known)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		WO 2000/050640	2000-08-31	EXACT LABORATORIES, INC.		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		

NONPATENT LITERATURE DOCUMENTS

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				Application Number	15/634,607
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				Art Unit	1634
				Examiner Name	WHISENANT
Sheet	2	of	2	*Attorney Docket Number*	EXCTD-35239.304

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Alimentary Pharmacology & Therapeutics

Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study

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SUMMARY

Background
Faecal occult blood testing is an established method of colorectal neoplasia screening. Guaiac-based tests are limited by poor patient compliance, low sensitivity, specificity and positive predictive value. Newer immunochemical-based tests, accurate but tedious, require a well-established laboratory set up. There is need for simpler immunochemical tests that can be performed at the out-patient clinic.

Aim
To compare the performance characteristics of a new bedside immunological test strip device with a sensitive Guaiac-based and established immunochemical test for detection of faecal occult blood in patients undergoing colonoscopy.

Methods
A total of 389 consecutive patients from four centres who were referred for colonoscopy also provided the stool samples for detection of occult blood without dietary restrictions. Stool tests performed were (i) Guaiac-based, (ii) immunochemical enzyme-linked immunosorbent assay and (iii) bedside immunochemical strip test.

Results
At the optimal threshold level, the sensitivity and specificity of the beside immunochemical strip test for detection of significant colorectal neoplasia (adenomas >1.0 cm and carcinomas) were 60% and 95%, respectively.

Conclusions
This bedside immunochemical strip test proved to be a simple, convenient, non-cumbersome and accurate tool with similar performance characteristics for detection of any bleeding lesion including colorectal neoplasia when compared with an established immunochemical faecal occult blood test.

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INTRODUCTION

Colorectal cancer (CRC) accounted for about 1 million new cases in 2002; its worldwide prevalence is only second to that of breast cancer. It is one of the leading causes of cancer death in western societies with 25-fold variation in its occurrence across the globe.[1] In Europe, there were estimated to be 376 400 new cases of CRC amounting to 203 700 deaths in 2004[2] while in the US there are expected to be 145 200 CRC registrations and 56 290 deaths this year.[3] It is estimated that about half of these deaths could have been prevented if patients would have undergone CRC screening.[4] It has been observed that most cases of sporadic CRC arise from pre-existing adenomatous polyps.

The metastatic CRC [stage IV tumour node metastasis (TNM)] has a dismal survival of <5% when compared with early CRC (stage I TNM).[5] The prevalence of the disease, with the proven ability of screening to reduce mortality makes population-wide screening of CRC a lucrative health goal. An ideal screening test must be non-invasive, acceptable to patients, simple, have high sensitivity and specificity, inexpensive, cost-effective, easy to implement across a large population and should be effective in reducing morbidity and mortality from CRC in the screened population.[6]

The most widely accepted non-invasive method for detecting CRC is faecal occult blood testing (FOBT). It is routinely used world over for CRC screening and surveillance and has been in use now for more than three decades. Screening in asymptomatic populations with FOBTs followed by colonoscopy if tested positive have reduced mortality rates by 15–33%.[7–10] However, sensitivity of FOBTs ranges from 26% to 69% for detecting CRC.[11–14]

There are two main types of commercially available FOBTs: 'Guaiac-based tests' in which Guaiac blue colour change occurs because of the pseudoperoxidase activity of haeme in the stool; 'immunochemical tests' which detect globin portion of human haemoglobin (Hb) in the stool using anti-Hb antibodies.[15] A major disadvantage of Guaiac tests is the fact that they are not specific for human blood and can produce false-positive results when meat, fruit, or vegetables containing peroxidase have been ingested. Also bleeding in the upper gastrointestinal (GI) tract secondary to aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) can produce false-positive Guaiac-based tests.[16] This may lead to contraindicate colonoscopic examinations thus increasing the cost and risk of screening programmes. False-negative tests may occur with ingestion of high doses of vitamin C, which will prevent detection of cancer and will provide a false sense of security to the patient. Because of these shortcomings Guaiac-based tests are being replaced by immunochemical faecal occult blood tests (IFOBTs), despite being more expensive.

The IFOBTs, which are widely used as screening tests in Japan, have been shown to be superior to the Guaiac-based tests in sensitivities, with similar specificity, as reported both in known CRC[17] and in asymptomatic populations.[13, 18] This is because IFOBTs do not detect blood from the upper GI tract, do not react with plant peroxidases and animal Hb. Thus, there is no need of dietary or medical restriction. One of the studies has shown 66% increase in patient participation with the elimination of dietary restrictions.[19] Furthermore, sensitivity of IFOBTs is high for adenomas,[17] intramucosal cancer[20] and Dukes A cancer.[21] On the basis of these reports, it is expected that screening with IFBOTs may provide larger effects on the incidence reduction of advanced CRC when compared with the Guaiac-based tests. However, most IFOBTs require a suitable well-equipped laboratory, i.e. an enzyme-linked immunosorbent assay (ELISA) reader, limiting their widespread use because of logistics and higher costs.

Colonoscopy is considered the gold standard for CRC screening. But it is expensive, requires skilled endoscopist and may lead to life-threatening complications like perforation (one in 300 procedures).[22] However, complication rate varies in different studies and one of the recent studies in which more than 22 000 patients underwent screening colonoscopies (only diagnostic) by experienced endoscopists without any major complication or mortality.[23]

In a recent pilot study, we demonstrated a new immunological test strip device (bedside IFOBT) for the rapid, quantitative detection of FOB (Prevent ID CC, Preventis GmbH, Bensheim, Germany) and compared it with a widely used Guaiac-FOBT keeping ELISA-IFOBT as the gold standard. We showed that this bedside IFOBT is much more sensitive (76% vs. 30%) for detection of FOB than Guaiac-based test whereas specificity of both tests were comparable.[24] However, we did not perform colonoscopy in these patients.

In the present prospective study, we have evaluated the performance characteristics of this new bedside test for detection of FOB in patients undergoing colonoscopy. Experienced endoscopists carefully examined

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Exact Sciences Ex. 2003, Page 875 of 1394

the entire large bowel, recorded and reported the presence of any bleeding lesion that could cause positive FOBT. If any such lesion was found it was biopsied or snared if indicated. We also compared it with another established IFOBT (ELISA) and a sensitive Guaiac-based FOBT.

Our study is peculiar and different from previous studies in that the patient population was not only of CRC screening patients but also constituted the patients referred to or admitted with colonic symptoms and many of them had known clinical diagnosis. Thus, we evaluated performance characteristics of FOBT not only in detecting neoplasia but also in detecting presence of microscopic faecal blood whether it was because of neoplasia, inflammatory bowel disease (IBD) or dysentery.

MATERIAL AND METHODS

Ethics

The study protocol was approved by the ethics committee of Frankfurt University Hospital. Written informed consent was obtained from all participants before entering the study.

Patients and healthy controls

Patients from four different centres who were referred for colonoscopy were invited to participate in the study. There were 237 patients who either had known clinical diagnosis (e.g. IBD) or were symptomatic suggestive of colonic disease while as 150 healthy patients underwent CRC screening. Patients with active GI bleeding, menstruation and past history of total colectomy were not included in the study.

Patients given appointments for colonoscopy were asked to provide one stool sample for detecting faecal blood. No dietary restrictions were advised to the patients. Stool sample was collected before starting the bowel preparation in a sterile box for Guaiac and immunological tests whereas for the bedside IFOBT we provided patients with a simple stool-collecting device (see below). Stool samples of these patients with or without pathological findings were tested. In a standard questionnaire patient's clinical history was recorded.

After standard bowel wash colonoscopies were performed by the endoscopists, who were not aware of FOBT findings, till the caecum using the standard colonoscopes. Patients who were poorly prepared or in whom caecum could not be reached were not included in the study. Histology was obtained from the routine biopsies, polypectomies and/or from surgery. Adenomas were classified as large (>10 mm) or small (<10 mm) as judged during colonoscopies using biopsy forceps as measure to assess polyp size. All data were collected prospectively and recorded on standardized questionnaire forms. If a neoplastic obstructive lesion was found during colonoscopies, it was biopsied and the patient was included in the study despite colonoscopy not being complete.

Faecal occult blood tests

Stool samples were applied to the three windows of a Guaiac-FOBT Haemoccult (Beckman Coulter, Inc., Fullerton, CA, USA) and further processed without rehydration as indicated by the manufacturer. On an especially Guaiac resin impregnated filter paper stool sample was placed. Once it had dried alcohol-stabilized hydrogen peroxide was put on the paper. A positive test was indicated by colour change to blue after 60 s.

A human Hb ELISA (Immundiagnostik AG, Bensheim, Germany) was used as a highly sensitive IFOBT. This test is based on Hb antibodies-precoated microtitre plates. To 100 mg of faeces wash buffer was added, mixed and centrifuged. About 100 L of this solution was incubated on the microtitre plates precoated with anti-Hb antibodies. After washing anti-Hb a peroxidase-labelled antibody was added, and was incubated for 1 h. Then, the substrate was added and incubated for 20 min and finally the stop solution was added so the colour changed from blue to yellow. Absorption was determined with an ELISA reader at 450 nm against reference value. According to the manufacturer a cut-off level of ≤10 g Hb/mL of stool was used.

An immunological FOB test strip device (Prevent ID CC) was used. It is a one-step immunochromatographic assay for rapid quantitative detection of human faecal blood. One of its major advantages, which should make it more acceptable to the patients, is the simplicity and non-messy sample collection. Figure 1 demonstrates the convenience of this test. The stick of the sample collection device was sticked into the faeces (approximately 2 cm), it was then inserted into the collection device containing an extraction buffer solution and the assembly was shaken thoroughly. This procedure was repeated three times sampling the

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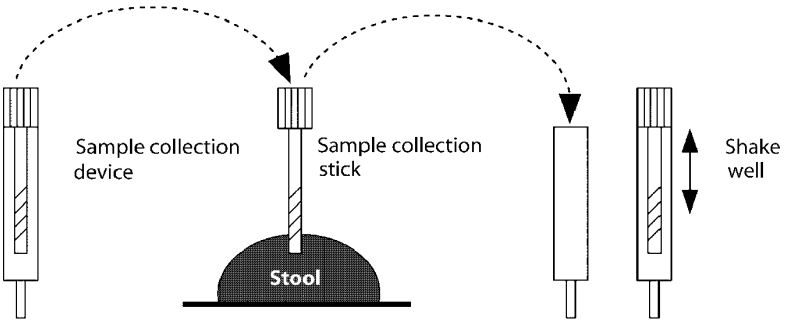


Figure 1. Sample collection for the bedside immunochemical faecal occult blood test (IFOBT; immunological test strip device; Prevent ID CC).

faeces from different sites. If the test was not performed within 1 day of sample collection it was stored at 2–8 C (as per the manufacturer's instructions). Two drops of this extracted sample were loaded into the sample opening of the test strip device, liquid faecal extract flows from the sample opening of the test device through the conjugate-release pad.

Principally it is a sandwich immunoassay in which gold-labelled anti-Hb antibodies bind to Hb (if present) in the sample. These complexes migrate over the test membrane until it passes the test zone, which consists of immobilized gold-labelled antibody–Hb complex and generates a violet band within 10 min (Figure 2a,b). To prevent false-negative an internal (control) line consisting of anti-IgG antibody has been added which indicates that the test has run properly. It has a very high sensitivity and can detect human Hb up to 10 g/mL.

The bedside FOBT was easy to use, and results did not depend on operator experience. All the three FOBTs were performed and reported by an experienced technician who was blinded to the patient's clinical profile.

Statistical analysis

For the statistical comparison of the non-parametrically distributed data, the Kruskall–Wallis ANOVA test was used (STATISTICA Version 5.0, StatSofts Inc., Tulsa, OK, USA). Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values of all the three FOBTs were determined.[25] Specificity was calculated for CR neoplasia as a whole group and not separately for adenoma and CRC. We used chi-square and Fisher's exact tests to compare differences in proportions amongst the tests. The *P*-values of <0.05 were considered as statistically significant (for sensitivity and specificity). Methods for proportions were used to calculate 95% confidence interval (CI).

RESULTS

A total of 387 consecutive patients underwent 407 tests, i.e. colonoscopies and FOBTs simultaneously. In 14 patients tests were repeated twice while in three patients they were repeated thrice. All the patients with repeat test were known cases of IBD. Their age ranged from 5 to 96 years (median age: 51 years). There was slight preponderance of females; 186 males and 201 females. The study duration was from January 2002 to December 2004. The major indications for colonoscopy were CRC screening, IBD, clinical suspicion of CR neoplasia or IBS, unexplained anaemia or diarrhoea, positive FOBT, etc. Comparative results of the test characteristics of asymptomatic screened patients and symptomatic patients are shown in Table 1. About 54 patients had evidence of CRC on colonoscopy (Table 2), the sensitivity of these tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively (Table 3). Of 18 adenomas diagnosed, six were large (>10 mm) and 12 were small, i.e. <10 mm. The sensitivity of faecal Hb for any neoplasia (CR carcinoma plus large adenomas) was 29.1%, 59.7%, and 63.8% respectively (Table 3). Of 164 patients with normal colonic mucosa, 16 (9.7%) were positive with the Guaiac test, nine (5.5%) with the bedside IFOBT, whereas six (3.7%) were found positive with ELISA-IFOBT, i.e. false-positive.

About 141 (34.6%) patients revealed a lesion (bleeding) on colonoscopy which is fairly high when compared with screening population. This is because, ours was a hospital-based study, and mostly included symptomatic patients unlike the screening studies that usually includes asymptomatic healthy individuals. Active bleeding colonic lesions were found in 67 (43.2%) of total 155 IBD patients examined, 29 with

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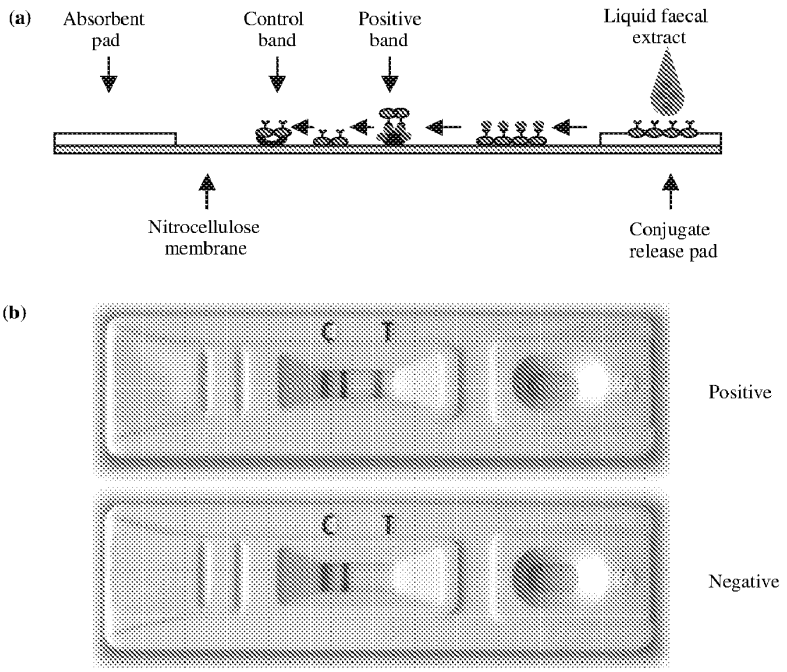


Figure 2. (a) Principle of the bedside immunochemical faecal occult blood test (IFOBT; immunological test strip device). After binding of faecal haemoglobin (blue dots) by antihaemoglobin antibodies, immuncomplexes are detected by a second nitrocellulose membrane-coupled antibody, resulting in the appearance of a positive band. The formation of a control band indicates the correct function of the test and unimpeded flow of faecal extracts through the device. (b) Photograph of Prevent ID CC test strips from a faecal specimen negative or positive for faecal occult blood.

Table 1. Test characteristics in screening and symptomatic patients

Diagnosis	*N*	Positive Guaiac-based test, *N* (%)	Positive bedside IFOBT, *N* (%)	Positive ELISA-IFOBT, *N* (%)
Screening patients				
Colorectal neoplasia*	6	2 (33.3)	4 (66.7)	4 (66.7)
Normal	150	17 (11.4)	8 (5.3)	5 (3.3)
Total	156	19 (12.2)	12 (7.7)	9 (5.8)
Symptomatic patients/patients with known disease				
Colorectal neoplasia*	66	19 (28.8)	39 (59)	42 (63.6)
IBD	155	39 (25.2)	68 (43.9)	68 (43.9)
Others	30	3 (10)	3 (10)	3 (10)
Total	251	61 (24.3)	110 (43.8)	113 (45.0)

* CRC + large adenomas.
CRC, colorectal cancer; IFOBT, immunochemical faecal occult blood test; IBD, inflammatory bowel disease; ELISA, enzyme-linked immunosorbent assay.

ulcerative colitis (UC) and 38 with Crohn's disease (CD). About 88 patients had inactive IBD (i.e. no evidence of bleeding lesion during colonoscopy), 22 UC and 66 CD. Two patients with pseudomembranous colitis had all the three FOBTs-positive in them. Sensitivity and specificity of rapid bedside test was found to be statistically significant in detecting any bleeding lesion (except adenomas) when compared with Guaiac-based tests.

DISCUSSION

The CRC and adenomas bleed intermittently[26] and positivity of FOBTs depend on degree of blood loss. Generally 2 mL of blood is necessary in stool to produce positive Guaiac-based tests.[5] When compared with CRC, adenomas bleed less frequently and also the bleeding is small in amount,[27] i.e. why sensitivity to detect them is lower. Also in the present study, the

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Table 2. Endoscopic diagnoses in 407 consecutive patients

Diagnosis	Total number	Positive Guaiac-based test, *N* (%)	Positive bedside IFOBT, *N* (%)	Positive ELISA-IFOBT, *N* (%)
Normal*	164	16 (9.7)	9 (5.5)	6 (3.7)
Colorectal cancer	54	20 (37.0)	40 (74.0)	42 (77.7)
Adenoma	18	1 (5.6)	3 (16.7)	4 (22.2)
Large (>10 mm)	6	1 (16.6)	2 (33.3)	3 (50.0)
Small (<10 mm)	12	0 (0)	0 (0)	0 (0)
IBD	155	39 (25.2)	68 (43.9)	68 (43.9)
UC	51			
Active	29	14 (48.3)	27 (93.1	28 (96.6)
Inactive	22	2 (9.1)	1 (4.5)	1 (4.5)
CD	104			
Active	38	16 (42.1)	36 (94.7)	36 (94.7)
Inactive	66	7 (10.6)	4 (6.0)	3 (4.5)
Diverticulosis	10	2 (20)	0 (0)	0 (0)
Pseudomembranous colitis	2	2 (100)	2 (100)	2 (100)
Hyperplastic polyps	4	0 (0)	0 (0)	0 (0)

* Includes 13 patients with irritable bowel syndrome and 15 patients with unexplained diarrhoea.
IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; IFOBT, immunochemical faecal occult blood test; ELISA, enzyme-linked immunosorbent assay.

Table 3. Sensitivities, specificities, PPV and NPV of three FOBTs in the different diagnostic groups

Parameters	Guaiac-based test	Bedside IFOBT	ELISA-IFOBT
Sensitivity (%)			
Adenoma	5.56 (0.14–27.29)	18.9 (3.58–41.42)	22.2* (6.41–47.64)
Cancer	37.0 (24.29–51.26)	74.0* (60.35–85.04)	77.7* (64.40–87.96)
Cancer + adenoma	29.1 (19.05–41.07)	59.7* (47.50–71.12)	63.8* (51.71–74.88)
Ulcerative colitis	48.2 (29.45–67.47)	93.1 (77.23–99.15)	96.5 (82.24–99.91)
Crohn's disease	42.1 (26.31–59.18)	94.7* (82.25–99.36)	94.7* (82.25–99.36)
Specificity (%)			
Cancer + adenoma	90.2 (84.64–94.32)	94.5 (89.84–97.46)	96.3* (92.21–98.65)
Ulcerative colitis	90.9 (70.84–98.88)	95.4* (77.16–99.88)	95.4 (77.16–99.88)
Crohn's disease	89.3 (79.36–95.63)	93.9* (85.20–98.32)	95.4* (87.29–99.05)
PPV (%)			
Adenoma	5.9 (0.15–28.69)	25.0 (5.49–57.19)	40.0 (12.16–73.76)
Cancer	55.6 (38.10–72.06)	81.6 (67.98–91.24)	87.5 (74.75–95.27)
Cancer + adenoma	56.7 (39.49–72.90)	82.6 (69.67–91.77)	88.4 (76.56–95.65)
Ulcerative colitis	87.5 (61.65–98.45)	96.4 (81.65–99.91)	96.6 (82.24–99.91)
Crohn's disease	69.6 (47.08–86.79)	90.0 (76.34–97.21)	92.3 (79.13–98.38)
NPV (%)			
Adenoma	89.7 (84.02–93.88)	91.2 (85.86–94.98)	91.9 (86.72–95.48)
Cancer	81.3 (74.89–86.70)	91.7 (86.49–95.40)	92.9 (87.99–96.30)
Cancer + adenoma	74.4 (67.72–80.28)	84.2 (78.16–89.18)	85.9 (79.99–90.56)
Ulcerative colitis	57.1 (39.35–73.68)	91.3 (71.96–98.93)	95.4 (77.16–99.88)
Crohn's disease	72.8 (61.81–82.13)	96.9 (89.16–99.62)	96.7 (89.32–99.63)

* $P < 0.05$ compared with Guaiac-based test.
Values in parentheses indicate 95% CI.
IFOBT, immunochemical faecal occult blood test; PPV, positive predictive value; NPV, negative predictive value; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval.

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Exact Sciences Ex. 2003, Page 879 of 1394

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sensitivity and specificity of all the three FOBTs performed were lowest for detecting adenomas when compared with all the other lesions.

The results of IFOBT performed on 1 day of stool sample has been shown to be almost as specific as Guaiac-based testing performed on 3 days of stool sample.[21] We have also used 1-day stool sample for conducting FOBTs. Faecal Hb loses its antigenicity at room temperature mostly because of its degradation by faecal flora,[28] so we stored stool samples at 4 C before testing.

One of the studies has demonstrated an increase in sensitivity by 7.4% but worsening of specificity by 7.3% with rehydration of stool sample with Guaiac-based tests.[7] We also did not rehydrate the stool sample before performing the Guaiac-based test. There is a broad range of variation in the sensitivity of Guaiac-based tests. According to Ahlquist and Shuber in a review of eight prospective Guaiac-based tests against colonoscopy, in screening population, showed that sensitivity for diagnosing CRC was 26% while for large adenomas it was 12%.[29] We observed the sensitivity of Guaiac-based test for detecting CRC to be 37% and for large adenomas 16.6%. Although the specificity for Guaiac-based tests lies in the range of 88–98% actually it is misleading as false-positives outnumber the true positives. Only one in 10 subjects with a positive Guaiac-based test harbour a CRC.[15] Thus, despite high specificity PPV is low.

The clinical value of technically comparable test strip devices for the rapid qualitative detection of other human proteins, e.g. cardiac troponin T or prostate-specific antigen, has already been proven.[30, 31] Here, we have utilized the same principle for detection of FOB using an immunological test strip device.

We found the performance characteristics of the bedside IFOBT in detecting CRCs to be comparable with ELISA-IFOBT. The sensitivity of this new bedside IFOBT for diagnosing CR neoplasia was 59.7% vs. 63.8% (P 0.29) for ELISA-IFOBT whereas the specificity of these two tests were 94.5 vs. 96.3 (P 0.38) respectively. Thus, the sensitivity and specificity of bedside IFOBT was lower than that of ELISA-IFOBT, but not statistically significant.

The sensitivity of both IFOBTs in the present study was in the same range or even better than that reported by other authors, and far higher than the values reported for Guaiac tests without rehydration.[32, 33] A large screening study revealed sensitivity of IFOBT for detecting advanced neoplasia and cancer to be 27% and 66% respectively.[23]

Upon comparison of rate of positive CR neoplasia in earlier large studies we noticed that the positivity rate of FOBT varied significantly (Table 4). These differences could be because of varied study populations and also inherent biases of these studies. As some of them were conducted in asymptomatic screening populations whereas others were performed in patients who were diseased. Also it is well evident that there is a wide range of variation in the rate of positivity of FOBT in detecting CR neoplasia, e.g. the range of HemOccult positivity is 47–84% while that of HemeSelect is 49–91%. Immunochemical tests were shown to be more sensitive than Guaiac-based tests.

In a study using a simple brush sampling technique for collection of stool for immunochemical 'Insure FOBT' demonstrated improved compliance by 66%. About 82% patients preferred brush over the

Table 4. Comparison of positive FOBTs in patients with CR neoplasia (CRC and adenoma >10 mm; modified from Ref.[24])

Reference	Lesions	Rate of positive lesions* (%)				
		HO	SENSA	HS	ELISA-IFOBT	Bedside IFOBT
Ahlquist *et al.*[12]	102	19	–	–	–	–
St John *et al.*[44]	152	75	84	91	–	–
Gopalswamy *et al.*[45]	9	60	78	89	–	–
Allison *et al.*[13]	142	38	47	49	–	–
Greenberg *et al.*[34]	55	38	47	49	–	–
Our study	60	35	–	–	76	71

* Positive test in patients with lesion documented by endoscopy.
HO, HemOccult II; SENSA, HemOccult II SENSA; HS, HemSelect; IFOBT, immunochemical faecal occult blood test; CRC, colorectal cancer; ELISA, enzyme-linked immunosorbent assay.

Exact Sciences Ex. 2003, Page 880 of 1394

traditional sampling method using the spatula for performing FOBT.[19] Similarly in this bedside IFOBT also the sample preparation is very simple with minimal stool handling, so we hope that it will improve patient acceptability for FOBTs.

Specificity is an important determinant of the cost of FOB screening. Even small changes in test specificity result in large effects on the number of false-positives in a screening study, which are responsible for unnecessary colonoscopic examinations. In our study the specificity defined by false-positive results if a normal colonic mucosa and no other cause of GI bleeding were shown to be between 89.3% and 90.9% for the Guaiac test, 93.9% and 95.4% for bedside IFOBT and 95.4% and 96.3% for the ELISA-IFOBT which is comparable with earlier reports in the literature.

One peculiar feature of our study is that subjects with variety of symptoms and with suspected or known clinical or endoscopic findings were included and underwent these tests. As the real focus of our work was to evaluate the relative performance characteristics of each of these three tests in diagnosing positive FOB irrespective of clinical diagnosis (whether because of CR neoplasia or active IBD) unlike previous studies which had excluded patients with known clinical diagnosis-like IBD, etc.[34, 35] Non-malignant bleeding lesions were diagnosed in 69 of total 141 patients showing positive bleeding lesions on colonoscopy.

There are mainly two large reports in the literature mentioning diagnosis of IBD in patients undergoing FOBTs. Actually in these studies asymptomatic IBD were diagnosed upon performing colonoscopy in patients detected positive with FOBTs during CRC screening in large sample population.[36, 37]

On comparing the costs of these tests, Guaiac-based FOBT costs around 1 €, ELISA-FOBT cost 15–20 € whereas for bedside IFOBT one has to pay 3–5 € for each test performed.

Newer promising non-invasive methods for CRC screening include detection of stool DNA for mutations-like p53, K-ras, APC, BAT-26, etc. However, initial enthusiasm with these tests could not be realized in large trials comparing it with Guaiac-based tests.[38–41] The major drawbacks of it are lower sensitivities, exorbitant costs (up to 800$), compliance issue as patient needs to send an entire bowel movement with a minimum weight of 30 g to be sent to the laboratory, which may not be acceptable to many patients and last but not the least anxiety associated with false-positive tests.

The inherent limitations of our study include, at first that a highly selected population undergoing colonoscopy for several 'high-risk' indications were included, and so care should be taken when applying our results to an average-risk asymptomatic population. Nevertheless, we feel that useful information was obtained about the feasibility and relative specificity of this new bedside IFOBT in our population. And secondly the use of endoscopy as the standard for the presence of bleeding lesion. Though colonoscopy is widely considered as the gold standard for detection of colonic bleeding lesions, miss rates of up to 6% in detection of adenomatous polyps are documented.[42, 43] This might have caused underestimation of results of FOBT in our study.

The biggest advantages of this new bedside IFOBT are its simplicity, rapidity (needs just about 10 min), convenience, no need of cumbersome ELISA readers and the fact that it can be performed at the patient bedside or in doctor's office by any health care personnel. Thus, performing FOBT may become as simple as doing serum blood glucose determination using glucometer. This advance should make screening for CRC an attainable goal and should increase patient compliance.

To conclude, FOBT is the only method of CRC screening whose efficacy has been established in randomized-controlled trials. We have shown that the new bedside IFOBT, is more specific and appears to be more accurate than the Guaiac-based HemOccult test for the detection of significant colonic bleeding lesions including CR neoplasia. Although this constitutes a pilot study only, it provides some guidance as to which type of FOBT may be appropriate for large-scale screening programmes. Future larger-scale screening studies are required to evaluate it over a large asymptomatic sample size.

CONFLICT OF INTEREST

The authors have no conflicts of interest with the device manufacturers.

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Exact Sciences Ex. 2003, Page 881 of 1394

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REFERENCES

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74–108.
2 Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. *Ann Oncol* 2005; **16**: 481–8.
3 Jemal A, Murray T, Ward E, *et al.* Cancer statistics, 2005. *CA Cancer J Clin* 2005; **55**: 10–30.
4 Walsh JM, Terdiman JP. Colorectal cancer screening: scientific review. *JAMA* 2003; **289**: 1288–96.
5 Bresalier RS. Malignant neoplasms of the large intestine. In: Feldman, M, Friedman, LS, Sleisinger, MH, eds. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management.* Philadelphia, USA: W.B. Saunders, 2002: 2215–56.
6 Allison JE. Review article: Faecal occult blood testing for colorectal cancer. *Aliment Pharmacol Ther* 1998; **12**: 1–10.
7 Mandel JS, Bond JH, Church TR, *et al.* Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993; **328**: 1365–71.
8 Hardcastle JD, Chamberlain JO, Robinson MHE, *et al.* Randomised controlled trial of faecal occult-blood screening for colorectal cancer. *Lancet* 1996; **348**: 1472–7.
9 Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996; **348**: 1467–71.
10 Faivre J, Dancourt V, Lejeune C, *et al.* Reduction in colorectal cancer mortality by fecal occult blood screening in a French controlled study. *Gastroenterology* 2004; **126**: 1674–80.
11 Allison JE, Feldman R, Tekawa IS. Hemoccult screening in detecting colorectal neoplasm: sensitivity, specificity, and predictive value. Long-term follow-up in a large group practice setting. *Ann Intern Med* 1990; **112**: 328–33.
12 Ahlquist DA, Wieand HS, Moertel CG, *et al.* Accuracy of fecal occult blood screening for colorectal neoplasia. A prospective study using Hemoccult and HemoQuant tests. *JAMA* 1993; **269**: 1262–7.
13 Allison JE, Tekawa IS, Ransom LJ, Adrain AL. A comparison of fecal occult-blood tests for colorectal-cancer screening. *N Engl J Med* 1996; **334**: 155–9.
14 Lieberman DA, Weiss DG. One-time screening for colorectal cancer with combined fecal occult-blood testing and examination of the distal colon. *N Engl J Med* 2001; **345**: 555–60.
15 Ouyang DL, Chen JJ, Getzenberg RH, Schoen RE. Noninvasive testing for colorectal cancer: a review. *Am J Gastroenterol* 2005; **100**: 1393–403.
16 Macrae FA, St John DJ, Caligiore P. Optimal dietary conditions for Hemoccult testing. *Gastroenterology* 1982; **82**: 899–903.
17 Sieg A, Thoms C, Luthgens K, John MR, Schmidt-Gayk H. Detection of colorectal neoplasms by the highly sensitive hemoglobin-haptoglobin complex in feces. *Int J Colorectal Dis* 1999; **14**: 267–71.
18 Petrelli N, Michalek AM, Freedman A, Baroni M, Mink I, Rodriguez-Bigas M. Immunochemical versus Guaiac occult blood stool tests: results of a community-based screening program. *Surg Oncol* 1994; **3**: 27–36.
19 Cole SR, Young GP, Esterman A, Cadd B, Morcom J. A randomised trial of the impact of new faecal haemoglobin test technologies on population participation in screening for colorectal cancer. *J Med Screen* 2003; **10**: 117–22.
20 Saito H, Soma Y, Koeda J, *et al.* Reduction in risk of mortality from colorectal cancer by fecal occult blood screening with immunochemical hemagglutination test. A case-control study. *Int J Cancer* 1995; **61**: 465–9.
21 Castiglione G, Zappa M, Grazzini G, *et al.* Immunochemical vs Guaiac faecal occult blood tests in a population-based screening programme for colorectal cancer. *Br J Cancer* 1996; **74**: 141–4.
22 Nelson DB, McQuaid KR, Bond JH, Lieberman DA, Weiss DG, Johnston TK. Procedural success and complications of large-scale screening colonoscopy. *Gastrointest Endosc* 2002; **55**: 307–14.
23 Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T, Shiratori Y. A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 2005; **129**: 422–8.
24 Trojan J, Povse N, Schroder O, Stein J. A new immunological test strip device for the rapid, qualitative detection of faecal occult blood. *Z Gastroenterol* 2002; **40**: 921–4.
25 Hulley SB, Cummings SR. *Designing Clinical Research.* Baltimore, USA: Williams & Wilkins, 1988.
26 Ahlquist DA, McGill DB, Fleming JL, *et al.* Patterns of occult bleeding in asymptomatic colorectal cancer. *Cancer* 1989; **63**: 1826–30.
27 Ahlquist DA, McGill DB, Schwartz S, Taylor WF, Owen RA. Fecal blood levels in health and disease. A study using HemoQuant. *N Engl J Med* 1985; **312**: 1422–8.
28 Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing. *Jpn J Cancer Res* 1996; **87**: 1011–24.
29 Ahlquist DA, Shuber AP. Stool screening for colorectal cancer: evolution from occult blood to molecular markers. *Clin Chim Acta* 2002; **315**: 157–68.
30 Lein M, Jung K, Schnorr D, Henke W, Loening SA. Strip test for the quick detection of increased concentrations of prostate-specific antigen in blood. *Eur J Clin Chem Clin Biochem* 1996; **34**: 511–4.
31 Muller-Bardorff M, Rauscher T, Kampmann M, *et al.* Quantitative bedside assay for cardiac troponin T: a complementary method to centralized laboratory testing. *Clin Chem* 1999; **45**: 1002–8.
32 Rozen P, Knaani J, Samuel Z. Eliminating the need for dietary restrictions when using a sensitive Guaiac fecal occult blood test. *Dig Dis Sci* 1999; **44**: 756–60.
33 Rozen P, Knaani J, Samuel Z. Comparative screening with a sensitive Guaiac and specific immunochemical occult blood test in an endoscopic study. *Cancer* 2000; **89**: 46–52.
34 Greenberg PD, Bertario L, Gnauck R, *et al.* A prospective multicenter evaluation of new fecal occult blood tests in patients undergoing colonoscopy. *Am J Gastroenterol* 2000; **95**: 1331–8.
35 Wong BC, Wong WM, Cheung KL, *et al.* A sensitive Guaiac faecal occult blood test is less useful than an immunochemical test for colorectal cancer screening in a Chinese population. *Aliment Pharmacol Ther* 2003; **18**: 941–6.

Exact Sciences Ex. 2003, Page 882 of 1394

36 Howarth GF, Robinson MH, Jenkins D, Hardcastle JD, Logan RF. High prevalence of undetected ulcerative colitis: data from the Nottingham fecal occult blood screening trial. *Am J Gastroenterol* 2002; **97**: 690–4.

37 Sakata T, Niwa Y, Goto H, *et al.* Asymptomatic inflammatory bowel disease with special reference to ulcerative colitis in apparently healthy persons. *Am J Gastroenterol* 2001; **96**: 735–9.

38 Ahlquist DA, Skoletsky JE, Boynton KA, *et al.* Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000; **119**: 1219–27.

39 Woolf SH. A smarter strategy? Reflections on fecal DNA screening for colorectal cancer. *N Engl J Med* 2004; **351**: 2755–8.

40 Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704–14.

41 Allison JE. Colon Cancer Screening Guidelines 2005: the fecal occult blood test option has become a better fit. *Gastroenterology* 2005; **129**: 745–8.

42 Hixson LJ, Fennerty MB, Sampliner RE, McGee D, Garewal H. Prospective study of the frequency and size distribution of polyps missed by colonoscopy. *J Natl Cancer Inst* 1990; **82**: 1769–72.

43 Rex DK, Cutler CS, Lemmel GT, *et al.* Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997; **112**: 24–8.

44 St John DJ, Young GP, Alexeyeff MA, *et al.* Evaluation of new occult blood tests for detection of colorectal neoplasia. *Gastroenterology* 1993; **104**: 1661–8.

45 Gopalswamy N, Stelling HP, Markert RJ, Maimon HN, Wahlen SD, Haddy RI. A comparative study of eight fecal occult blood tests and HemoQuant in patients in whom colonoscopy is indicated. *Arch Fam Med* 1994; **3**: 1043–8.

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Application Number	15/634,607	Filing Date	2017-06-27	Docket Number (if applicable)	EXCTD-35239.304	Art Unit	1634
First Named Inventor	JOOST LOUWAGIE			Examiner Name	WHISENANT		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	*Ex Parte* Reexamination of U.S. Patent No. 11,634,781
Issued:	April 25, 2023
App. Ser. No.:	17/936,335
Filing Date:	September 28, 2022
Named Inventor:	Joost Louwagie
Title:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Mail Stop: *Ex Parte* Reexam
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR EX PARTE REEXAMINATION UNDER 37 C.F.R. § 1.510

Dear Commissioner:

Ex parte reexamination under 35 U.S.C. §§ 302-307 and 37 C.F.R. § 1.510 is requested of U.S. Patent No. 11,634,781 ("the '781 Patent"). Reexamination is requested of claims 1-20 as these claims are anticipated and/or obvious in view of the prior art identified in the citation of prior art under 37 C.F.R. §1.501 submitted herewith. The Rule 501 citation includes a PTO form PTO/SB/08a and copies of all prior art relied upon.

Requestor, as confirmed by the undersigned attorney, hereby certifies that the statutory estoppel provisions of 35 U.S.C. § 315(e)(1) and 35 U.S.C. § 325(e)(1) do not prohibit Requester from filing this *ex parte* reexamination request.

Table of Contents

Page

I. Identification of Claims for Which Reexamination is Requested 1

II. Overview of the '781 Patent 1

A. Summary of the Independent Claims 1

B. The '781 Patent Prosecution History Lacks Any Prior Art Rejections 1

III. The Level of Ordinary Skill in the Art 2

IV. Claim Construction 3

A. Claims 1-20 3

B. Claims 5-11 5

V. Other Supporting Evidence 5

A. Declaration of Mr. Anthony P. Shuber, MS 5

VI. Statement Pointing Out Each Substantial New Question of Patentability 6

A. Lapidus '178 13

B. Lapidus '650 14

C. Lapidus '178 in view of De Luca and Olson 14

D. Lapidus '650 in view of De Luca and Olson 15

E. Ahlquist in view of De Luca and Olson 15

F. O'Neill in view of De Luca and Olson 15

G. Lapidus '178 in view of De Luca, Olson, and Leung 16

H. Lapidus '650 in view of De Luca, Olson, and Leung 16

I. Ahlquist in view of De Luca, Olson, and Leung 16

J. O'Neill in view of De Luca, Olson, and Leung 17

K. Lapidus '178 in view of De Luca, Olson, and Hoepffner 17

L. Lapidus '650 in view of De Luca, Olson, and Hoepffner 17

M. Ahlquist in view of De Luca, Olson, and Hoepffner 18

N. O'Neill in view of De Luca, Olson, and Hoepffner 18

VII. Detailed Explanation under 37 C.F.R. § 1.510(b) 18

A. Independent Claim 1 23

B. Dependent Claim 2 108

C. Dependent Claim 3 114

D. Dependent Claim 4 185

E. Dependent Claim 5 196

F. Dependent Claim 6 201
G. Dependent Claim 7 204
H. Dependent Claim 8 207
I. Dependent Claim 9 211
J. Dependent Claim 10 217
K. Dependent Claim 11 221
L. Dependent Claim 12 225
M. Dependent Claim 13 237
N. Dependent Claim 14 210
O. Dependent Claim 15 250
P. Dependent Claim 16 260
Q. Dependent Claim 17 265
R. Dependent Claim 18 269
S. Dependent Claim 19 273
T. Dependent Claim 20 278
VIII. Conclusion 282

Exhibits

Exhibit 1001: U.S. Patent No. 11,634,781 ("the '781 Patent")

Exhibit 1002: U.S. Patent No. 5,952,178 to Lapidus et al. ("Lapidus '178")

Exhibit 1003: U.S. Patent No. 5,741,650 to Lapidus et al. ("Lapidus '650")

Exhibit 1004: Ahlquist et al., "Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia," *Ann Intern Med.*, 149(7):441-W81 (published in final form October 7, 2008) ("Ahlquist")

Exhibit 1005: Patent Cooperation Treaty (PCT) Publication No. WO 2005/014154 to O'Neill ("O'Neill")

Exhibit 1006: European Patent Application Publication No. EP1366715A1 to De Luca et al. ("De Luca")

Exhibit 1007: Olson et al., "DNA Stabilization Is Critical for Maximizing Performance of Fecal DNA-Based Colorectal Cancer Tests," *Diagn Mol Pathol*, 14:183–191 (published September 2005) ("Olson")

Exhibit 1008: Leung et al., "Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of Patients With Colorectal Cancer or Polyps," *Am J Gastroenterol*, 102:1070–1076 (2007) ("Leung")

Exhibit 1009: Hoepffner et al., "Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study," *Aliment Pharmacol Ther*, 23, 145–154 (published 2006) ("Hoepffner")

Exhibit 1010: U.S. Patent No. 4,859,610 to Maggio ("Maggio")

Exhibit 1011: U.S. Patent No. 5,196,167 to Guandagno et al. ("Guandagno")

Exhibit 1012: U.S. Patent No. 5,137,806 to LeMaistre et al. ("LeMaistre")

Exhibit 1013: U.S. Patent No. 5,527,676 to Vogelstein et al. ("Vogelstein")

Exhibit 1014: Declaration of Mr. Anthony P. Shuber, MS

I. Identification of Claims for Which Reexamination is Requested

In accordance with 37 C.F.R. § 1.510, reexamination of claims 1-20 of the '781 Patent is requested, in view of the references cited herein.

II. Overview of the '781 Patent

A. Summary of the Independent Claims

The '781 Patent issued on April 25, 2023 and includes one independent claim. As discussed below, independent claim 1 and its respective dependent claims are known in the prior art.

Claim 1 is the sole independent claim as shown below:

1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:

a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;

b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;

c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and

d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.

B. The '781 Patent Prosecution History Lacks Any Prior Art Rejections

The application for the '781 Patent was filed by Exact Sciences Corporation on September 28, 2022. As shown on the face of the '781 Patent (Ex. 1001), the '781 Patent claims priority to i) U.S. Patent Application No. 15/634,607, filed June 27, 2017, ii) U.S. Patent Application No. 15/010,436, filed January 29, 2016, iii) U.S. Patent Application No. 13/147,570, filed March 12, 2012, iv) PCT International Application No. PCT/GB2010/000180, filed February 3, 2010, and v) U.S. Provisional Patent Application No. 61/149,581, filed February 3, 2009.

The Office examined the application that resulted in the '781 Patent under the pre-AIA "first to invent" provisions as stated in the prosecution history of the '781 Patent. Requestor does not concede that the '781 Patent should be afforded this status but provides the anticipation and obviousness assertions herein according to the pre-AIA "first to invent" provisions as determined by the Office. However, if the '781 Patent is subject to the AIA's "first inventor to file" provisions, then Requestor notes that each prior art reference cited herein would qualify as prior art under AIA 35 U.S.C. § 102(a)(1).

Notably, the prosecution history of the application that resulted in the '781 Patent is ***completely devoid*** of prior-art based rejections, other than a provisional nonstatutory double patenting rejection over a co-pending Exact Sciences application related to the '781 Patent (i.e., co-pending U.S. Patent Application No. 15/634,607) and a provisional nonstatutory double patenting rejection over the co-pending Exact Sciences application in view of Ohlsson et al. (*Br J Cancer*, 2006; 95(2):218–225). The provisional nonstatutory double patenting rejections were made in an Office Action issued on January 11, 2023. In response, the applicant filed a Terminal Disclaimer on January 24, 2023. Thereafter, the provisional nonstatutory double patenting rejections were withdrawn.

The application was subsequently allowed on February 16, 2023. On pages 2-3 of the Notice of Allowance, the Examiner provided a statement of reasons for allowance, the entirety of which is quoted below:

> Claim(s) 1-20 is/are deemed to be allowable in light of the paper(s) filed 23 JAN 2023 and the persuasive argument(s) therein.

The application subsequently issued as the '781 Patent on April 25, 2023. Ex. 1001. As discussed below, the claimed subject matter in the '781 Patent was known in the prior art and lacks novelty and/or would have been obvious to one of ordinary skill in the art.

III. The Level of Ordinary Skill in the Art

As set forth above, the '781 Patent issued from an application claiming priority from a provisional application filed on February 3, 2009. As such, the earliest possible effective filing date that could be accorded the '781 Patent is February 3, 2009. Requestor does not concede that any or all claims of the '781 Patent should be afforded an effective filing date of February 3, 2009, but has nevertheless treated this date as the effective filing date of all claims solely for purposes of this Request.

The relevant field of "the invention of the '781 Patent is the field of processing and evaluating fecal samples. Ex. 1014 at paragraph 18. A person of ordinary skill in this field:

> would have had at least a Bachelor of Science degree in Biology (or a similar major) and several years of experience in the processing of biological samples, such as fecal samples from a human. Such a person would have been well-acquainted with methods of collecting fecal samples, preserving proteins and/or nucleic acids, including hemoglobin, DNA, and RNA, and subsequently evaluating the fecal samples for proteins and/or nucleic acids, including hemoglobin, DNA, and RNA, as of February 3, 2009.

Id. at paragraph 20.

IV. Claim Construction

During reexamination, claims are given the broadest reasonable interpretation consistent with the specification and limitations in the specification are not read into the claims. MPEP § 2258.

A. Claims 1-20

1. The Preamble of Independent Claim 1 is Not a Limitation

Requestor respectfully submits that the preamble of claim 1 is not a limitation. The Federal Circuit has held:

> In general, a preamble limits the invention if it recites essential structure or steps, or if it is necessary to give life, meaning, and vitality to the claim. Conversely, a preamble is not limiting where a patentee defines a structurally complete invention in the claim body and uses the preamble only to state a purpose or intended use for the invention. No litmus test defines when a preamble limits claim scope. Some guideposts, however, have emerged from various cases discussing the preamble's effect on claim scope. For example, this court has held that Jepson claiming generally indicates intent to use the preamble to define the claimed invention, thereby limiting claim scope. Additionally, dependence on a particular disputed preamble phrase for antecedent basis may limit claim scope because it indicates a reliance on both the preamble and claim body to define the claimed invention. Likewise, when the preamble is essential to understand limitations or terms in the claim body, the preamble limits claim scope. Further, when reciting additional structure or steps underscored as important by the specification, the preamble may operate as a claim limitation. Moreover, clear reliance on the preamble during prosecution to distinguish the claimed invention from the prior art transforms the preamble into a claim limitation because such reliance indicates use of the preamble to define, in part, the claimed invention. Without such reliance, however, a preamble generally is not limiting when the claim body describes a structurally complete invention such that deletion of the

> preamble phrase does not affect the structure or steps of the claimed invention. Thus, preamble language merely extolling benefits or features of the claimed invention does not limit the claim scope without clear reliance on those benefits or features as patentably significant.

Catalina Mktg. Int'l, Inc. v. Coolsavings.com, Inc., 289 F.3d 801, 808–09 (Fed. Cir. 2002) (internal quotations and citations omitted).

The preamble of claim 1 of the '781 Patent does not recite any essential structure or steps and is not necessary to give life, meaning, or vitality to the claim. Instead, claim 1 defines a complete method in the claim body and uses the preamble only to state a purpose or intended use for the alleged invention. Further, claim 1 of the '781 Patent is not drafted in Jepson format. Additionally, the preamble of claim 1 of the '781 Patent does not establish dependence on a particular disputed preamble phrase for antecedent basis as there is no apparent reliance on both the preamble and claim body to define the claimed invention, nor is the preamble of claim 1 of the '781 Patent essential to understand limitations or terms in the claim body. The preamble of claim 1 of the '781 Patent does not recite additional structure or steps underscored as important by the specification. Finally, there is no clear reliance on the preamble of claim 1 of the '781 Patent during prosecution to distinguish the claimed invention from the prior art. In summary, the preamble of claim 1 of the '781 Patent merely extols benefits or features of the claimed invention and Requestor asserts that the preamble of claim 1 is not a limitation.

2. "Without Freezing" Refers to a Temperature or Temperature Range Above 0°C (32°F)

All claims require that a fecal sample is processed "without freezing." The ordinary meaning of "freezing" is "being at or below the temperature at which water freezes" according to Merriam-Webster's Medical Dictionary (*available at* https://www.merriam-webster.com/dictionary/freezing). The temperature at which water freezes is known to be 0°C (32°F). Ex. 1014 at paragraph 27. Therefore, any temperature above 0°C (32°F), or a temperature range that includes a temperature above 0°C (32°F), would be understood as "without freezing" by those of ordinary skill in the art. *Id.*

3. "At Home" Refers to a Location Other Than a Testing Facility

All claims require that the fecal sample is collected "at home." The '781 Patent uses this term primarily to distinguish the location of collection from the testing facility. For instance, the '781 Patent states that "[f]ollowing collection of the sample and suitable processing, the method may further comprise forwarding or otherwise delivering the removed portion of the collected faecal sample *to a laboratory* for performing step (a) of the method, as defined herein, on the removed portion of the collected faecal sample." *Id.* at column 18, lines 32-35 (emphasis added). Similarly, dependent claim 2 of the '781 Patent indicates that the method of claim 1 *further comprises* "delivering the [fecal sample collected at home] to a medical diagnostics laboratory." *Id.* at column 45, lines 39-44.

Therefore, a person of ordinary skill in the art would understood "at home", as used in the '781 Patent, to refer to a location other than the testing facility. Ex. 1014 at paragraph 28.

B. Claims 5-11

1. "Epigenetic Modification" is an Alteration in DNA

Regarding claims 5-11, the '781 Patent defines "epigenetic modification" such that it includes "any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene." Ex. 1001 at column 12, lines 35-39. For instance, the '781 patent describes that "[i]n specific embodiments, the epigenetic modification is methylation." *Id.* at column 12, line 46-47. This definition of "epigenetic modification" provided by the '781 Patent is consistent with the definition of "epigenetic modification" as understood by those of ordinary skill in the art. Ex. 1014 at paragraph 29.

V. Other Supporting Evidence

A. Declaration of Mr. Anthony P. Shuber, MS

The Declaration of Mr. Anthony P. Shuber, MS is being submitted with this Request as Exhibit 1014 to "explain the contents or pertinent dates of prior art patents or printed publications in more detail." See MPEP § 2258(I)(E). Mr. Shuber has extensive experience in the fields of diagnostics and applied genomics, including a Bachelor of Science degree in Biology and a Master of Science degree in Molecular and Developmental Biology from

Marquette University in Milwaukee, Wisconsin. *See* Mr. Anthony P. Shuber curriculum vitae (Ex. 1014).

Mr. Shuber's work in the field includes developing several DNA diagnostics tests as well as diagnostics in oncology, with a focus on the early detection of cancer. He is currently the Chief Innovation Officer at Harbinger Health. Mr. Shuber is a named inventor or author on several prior art references cited herein, including Ex. 1002, Ex. 1003, and Ex. 1007.

His declaration provides factual evidence regarding the level of skill in the art, the prior art references, the scope and content of the prior art, and technical information relevant to the '781 Patent, as well as explaining why the claimed invention would have been obvious to a person of ordinary skill in the art.

VI. Statement Pointing Out Each Substantial New Question of Patentability

This Section addresses the substantial new questions of patentability and is organized by cited references.

As explained above, the Office did not issue prior art-based rejections during the examination that resulted in the '781 Patent. Moreover, with the exception of O'Neill, ***none of the prior art references identified below and discussed herein were cited*** by the Patentee or by the Office in the course of examination that resulted in the '781 Patent. Although O'Neill was cited by the Patentee, it was not utilized in any prior-art based rejection during the examination that resulted in the '781 Patent.

Accordingly, the following prior art references (except O'Neill) are ***newly cited*** in this reexamination request and raise substantial new questions of patentability because they are highly relevant to the claims of the '781 Patent as shown below and have not been substantively considered by the USPTO:

Prior Art Citation	Ex. No.	Summary
U.S. Patent No. 5,952,178 to Lapidus et al. ("**Lapidus '178**")	1002	Lapidus '178 is a granted U.S. patent and discloses methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids. *Id.* at, *e.g.,* column 7, line 59 – column 9, line 50. Fecal samples are collected from a human subject,

		separated into two or more portions, and combined with buffers. Lapidus '178 was patented on September 14, 1999 and therefore is prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
U.S. Patent No. 5,741,650 to Lapidus et al. ("**Lapidus '650**")	1003	Lapidus '650 is a granted U.S. patent and discloses methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids. *Id.* at, *e.g.,* column 7, line 12 – column 8, line 25. Fecal samples are collected from a human subject, separated into two or more portions, and combined with buffers. Lapidus '650 was patented on April 21, 1998 and therefore is prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
Ahlquist et al., "Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia," *Ann Intern Med.*, 149(7):441-W81 (published in final form October 7, 2008) ("**Ahlquist**")	1004	**Ahlquist** is a journal article and discloses collection of stool and comparison of stool DNA and fecal blood testing for detection of colorectal neoplasia. *Id.* at Abstract. Fecal samples are collected from a human subject, separated into two or more portions, and analyzed for both nucleic acids and blood proteins. Ahlquist was published and publicly available at least as early as October 7, 2008. Ex. 1014 at paragraph 37. Therefore, Ahlquist is at least prior art under pre-AIA 35 U.S.C. § 102(a) against the challenged claims.
Patent Cooperation Treaty (PCT) Publication No. WO 2005/014154 to O'Neill ("**O'Neill**")	1005	**O'Neill** is a published PCT application and discloses a method of removing cellular material from the surface of a fecal stool in a container. *Id.* at Abstract. The patient collects a fecal sample at home by defecation directly into a sealable container and then a portion of the fecal sample

		(cellular material) is removed for analysis of DNA, RNA, proteins, and the like. O'Neill was published and publicly available on February 17, 2005, and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
European Patent Application Publication No. EP1366715A1 to De Luca et al. ("**De Luca**")	1006	**De Luca** is a published European patent application and discloses an extraction tube for collection of fecal samples and combination with a buffer. *Id.* at [0019]. The extraction tube contains a sealable cap and the tube is utilized for laboratory immunological tests, such as for qualitative or quantitative determination of hemoglobin. De Luca was published and publicly available on December 3, 2003, and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
Olson et al., "DNA Stabilization Is Critical for Maximizing Performance of Fecal DNA-Based Colorectal Cancer Tests," *Diagn Mol Pathol*, 14:183–191 (published September 2005) ("**Olson**")	1007	**Olson** is a journal article and discloses collection of stool and detection of fecal DNA. *Id.* at Abstract. Fecal samples are collected from a human subject and a stabilizing buffer was added to the samples. Addition of the stabilizing buffer was shown to provide a significant increase in recovery of DNA from fecal samples. *Id.* Olson was published and publicly available in September 2005. Ex. 1014 at paragraph 40. Therefore, Olson is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
Leung et al., "Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of	1008	**Leung** is a journal article and discloses collection of stool and detection of fecal DNA. *Id.* at Abstract. Fecal samples are collected from a human subject and analyzed for hypermethylated DNA via

Patients With Colorectal Cancer or Polyps," *Am J Gastroenterol*, 102:1070–1076 (2007) ("**Leung**")		PCR and for mRNA via RT-PCR. Leung was published and publicly available in 2007. Ex. 1014 at paragraph 41. Therefore, Leung is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
Hoepffner et al., "Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study," *Aliment Pharmacol Ther*, 23, 145–154 (published 2006) ("**Hoepffner**")	1009	**Hoepffner** is a journal article and discloses a comparison of characteristics of a new bedside immunological test strip device with a sensitive Guaiac-based and established immunochemical test for detection of faecal occult blood in patients. *Id.* at Abstract. The concentrations of hemoglobin in nanograms/milliliter (ng/ml) resulting in a positive blood test in fecal samples is discussed. Hoepffner was published and publicly available in 2006. Ex. 1014 at paragraph 42. Therefore, Hoepffner is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims.
U.S. Patent No. 4,859,610 to Maggio **("Maggio")**	1010	**Maggio** is a granted U.S. patent and discloses a device that is used for homogenizing and analyzing fecal samples. *Id.* at, *e.g.,* column 4, lines 7-8. Fecal sample and phosphate buffered saline are homogenized in a separate sealable container (i.e., vessel) that contains a cap and seal. Maggio was patented on August 22, 1989 and therefore is prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Maggio is cited herein as an evidentiary reference only.

This Request seeks reexamination based on the following proposed rejections that raise substantial new questions of patentability for all claims of the '781 Patent. The specific application of the newly cited prior art references to such claims is detailed in Section VIII:

Claim	Prior Art Rendering Claims Anticipated Under 35 USC § 102(b)
1	Lapidus '178 (Ex. 1002)
	Lapidus '650 (Ex. 1003)
2	Lapidus '178
	Lapidus '650
3	Lapidus '178
	Lapidus '650
4	Lapidus '178
	Lapidus '650
12	Lapidus '178
	Lapidus '650

Claim	Prior Art Combinations Rendering Claims Obvious Under 35 USC § 103(a)
1	Lapidus '178
	Lapidus '650
	Lapidus '178 in view of De Luca (Ex. 1006) and Olson (Ex. 1007)
	Lapidus '650 in view of De Luca and Olson
	Ahlquist (Ex. 1004) in view of De Luca and Olson
	O'Neill (Ex. 1005) in view of De Luca and Olson
2	Lapidus '178
	Lapidus '650
	Lapidus '178 in view of De Luca and Olson
	Lapidus '650 in view of De Luca and Olson
	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca and Olson
3	Lapidus '178
	Lapidus '650
	Lapidus '178 in view of De Luca and Olson
	Lapidus '650 in view of De Luca and Olson

	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca and Olson
4	Lapidus '178
	Lapidus '650
	Lapidus '178 in view of De Luca and Olson
	Lapidus '650 in view of De Luca and Olson
	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca and Olson
5	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca, Olson, and Leung
6	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca, Olson, and Leung
7	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
8	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
9	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
10	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung

	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
11	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
12	Lapidus '178
	Lapidus '650
	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
13	Lapidus '178 in view of De Luca, Olson, and Leung
	Lapidus '650 in view of De Luca, Olson, and Leung
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca, Olson, and Leung
14	Lapidus '178 in view of De Luca and Olson
	Lapidus '650 in view of De Luca and Olson
	Ahlquist in view of De Luca, Olson, and Leung
	O'Neill in view of De Luca and Olson
15	Lapidus '178 in view of De Luca and Olson
	Lapidus '650 in view of De Luca and Olson
	Ahlquist in view of De Luca and Olson
	O'Neill in view of De Luca and Olson
16	Lapidus '178 in view of De Luca, Olson, and Hoepffner (Ex. 1009)
	Lapidus '650 in view of De Luca, Olson, and Hoepffner
	Ahlquist in view of De Luca, Olson, and Hoepffner
	O'Neill in view of De Luca, Olson, and Hoepffner
17	Lapidus '178 in view of De Luca, Olson, and Hoepffner
	Lapidus '650 in view of De Luca, Olson, and Hoepffner

	Ahlquist in view of De Luca, Olson, and Hoepffner
	O'Neill in view of De Luca, Olson, and Hoepffner
18	Lapidus '178 in view of De Luca, Olson, and Hoepffner
	Lapidus '650 in view of De Luca, Olson, and Hoepffner
	Ahlquist in view of De Luca, Olson, and Hoepffner
	O'Neill in view of De Luca, Olson, and Hoepffner
19	Lapidus '178 in view of De Luca, Olson, and Hoepffner
	Lapidus '650 in view of De Luca, Olson, and Hoepffner
	Ahlquist in view of De Luca, Olson, and Hoepffner
	O'Neill in view of De Luca, Olson, and Hoepffner
20	Lapidus '178 in view of De Luca, Olson, and Hoepffner
	Lapidus '650 in view of De Luca, Olson, and Hoepffner
	Ahlquist in view of De Luca, Olson, and Hoepffner
	O'Neill in view of De Luca, Olson, and Hoepffner

Section VIII. provides a further explanation of each of the references discussed in this Section. As explained below, the prior art references relied on here disclose all of the limitations of claims 1-20 of the '781 Patent. In view of the prior art, the challenged claims are anticipated "if each and every element as set forth in the claim[s] is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, (Fed. Cir. 1987). Furthermore, in view of this prior art, and taking into account the knowledge, experience, and creativity of a person of ordinary skill in the art, such a person would have found the challenged claims to be "a predictable use of prior art elements according to their established functions," *KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 417 (2007), and therefore obvious. Requestor, therefore, respectfully submits that substantial new questions of patentability have been raised by the prior art cited herein.

A. Lapidus '178

Lapidus '178 (Ex. 1002) is a granted U.S. patent owned by Exact Sciences Corporation and discloses methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids. *Id.* at, *e.g.,* column 7, line 59 – column 9, line 50. Fecal samples are collected

from a human subject and were sealed in a container, separated into two or more portions, and combined with buffers. Lapidus '178 was patented on September 14, 1999 and therefore is prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Lapidus '178 was not cited during prosecution of the '781 Patent.

As discussed fully in Section VIII. below, Lapidus '178 anticipates the elements of certain claims. Thus, the technological teachings of Lapidus '178 alone raise a substantial new question of patentability at least with respect to claims 1, 2, 3, 4, and 12 of the '781 Patent.

B. Lapidus '650

Lapidus '650 (Ex. 1003) is a granted U.S. patent owned by Exact Sciences Corporation and discloses methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids. *Id.* at, *e.g.,* column 7, line 12 – column 8, line 25. Fecal samples are collected from a human subject and were sealed in a container, separated into two or more portions, and combined with buffers. Lapidus '650 was patented on April 21, 1998 and therefore is prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Lapidus '650 was not cited during prosecution of the '781 Patent.

As discussed fully in Section VIII. below, Lapidus '650 anticipates the elements of certain claims. Thus, the technological teachings of Lapidus '650 alone raise a substantial new question of patentability at least with respect to claims 1, 2, 3, 4, and 12 of the '781 Patent.

C. Lapidus '178 in view of De Luca and Olson

Lapidus '178 is discussed briefly above. De Luca (Ex. 1006) is a published European patent application and discloses an extraction tube for collection of fecal samples and combination with a buffer. *Id.* at [0019]. The extraction tube contains a sealable cap and the tube is utilized for laboratory immunological tests, such as for qualitative or quantitative determination of hemoglobin. De Luca was published and publicly available on December 3, 2003, and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. De Luca was not cited during prosecution of the '781 Patent.

Olson (Ex. 1007) is a journal article and discloses collection of stool and detection of fecal DNA. *Id.* at Abstract. Fecal samples are collected from a human subject and a stabilizing buffer was added to the samples. Addition of the stabilizing buffer was shown to provide a significant increase in recovery of DNA from fecal samples. *Id.* Olson was published and

publicly available in September 2005 and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Olson was not cited during prosecution of the '781 Patent.

As discussed fully in Section VIII. below, the combined teachings of Lapidus '178, De Luca, and Olson render certain claims to be obvious. Thus, the technological teachings of Lapidus '178, De Luca, and Olson raise a substantial new question of patentability at least with respect to claims 1-4 and 14-15 of the '781 Patent.

D. Lapidus '650 in view of De Luca and Olson

Lapidus '650, De Luca, and Olson are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Lapidus '650, De Luca, and Olson render certain claims to be obvious. Thus, the technological teachings of Lapidus '650, De Luca, and Olson raise a substantial new question of patentability at least with respect to claims 1-4 and 14-15 of the '781 Patent.

E. Ahlquist in view of De Luca and Olson

Ahlquist is a journal article and discloses collection of stool and comparison of stool DNA and fecal blood testing for detection of colorectal neoplasia. *See* Abstract. Fecal samples are collected from a human subject, separated into two or more portions, and analyzed for both nucleic acids and blood proteins. Ahlquist was published and publicly available at least as early as October 7, 2008 and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(a) against the challenged claims. Ahlquist was not cited during prosecution of the '781 Patent.

De Luca and Olson are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Ahlquist, De Luca, and Olson render certain claims to be obvious. Thus, the technological teachings of Ahlquist, De Luca, and Olson raise a substantial new question of patentability at least with respect to claims 1-6 and 15 of the '781 Patent.

F. O'Neill in view of De Luca and Olson

O'Neill (Ex. 1005) is a published PCT application and discloses a method of removing cellular material from the surface of a fecal stool in a container. *Id.* at Abstract. The patient collects a fecal sample at home by defecation directly into a sealable container and then a portion

of the fecal sample (cellular material) is removed for analysis of DNA, RNA, proteins, and the like. O'Neill was published and publicly available on February 17, 2005, and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. O'Neill was cited during prosecution of the '781 Patent but was not included in any prior art rejection.

De Luca and Olson are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of O'Neill, De Luca, and Olson render certain claims to be obvious. Thus, the technological teachings of O'Neill, De Luca, and Olson raise a substantial new question of patentability at least with respect to claims 1-4, 14, and 15 of the '781 Patent.

G. Lapidus '178 in view of De Luca, Olson, and Leung

The teachings of Lapidus '178, De Luca, and Olson are briefly described above.

Leung is a journal article and discloses collection of stool and detection of fecal DNA. *Id.* at Abstract. Fecal samples are collected from a human subject and analyzed for hypermethylated DNA via PCR and for mRNA via RT-PCR. Leung was published and publicly available in 2007 and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Leung was not cited during prosecution of the '781 Patent.

As discussed fully in Section VIII. below, the combined teachings of Lapidus '178, De Luca, Olson, and Leung render certain claims to be obvious. Thus, the technological teachings of Lapidus '178, De Luca, Olson, and Leung raise a substantial new question of patentability at least with respect to claims 5-13 of the '781 Patent.

H. Lapidus '650 in view of De Luca, Olson, and Leung

The teachings of Lapidus '650, De Luca, Olson, and Leung are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Lapidus '650, De Luca, Olson, and Leung render certain claims to be obvious. Thus, the technological teachings of Lapidus '650, De Luca, Olson, and Leung raise a substantial new question of patentability at least with respect to claims 5-13 of the '781 Patent.

I. Ahlquist in view of De Luca, Olson, and Leung

The teachings of Ahlquist, De Luca, Olson, and Leung are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Ahlquist, De Luca, Olson, and

Leung render certain claims to be obvious. Thus, the technological teachings of Ahlquist, De Luca, Olson, and Leung raise a substantial new question of patentability at least with respect to claims 7-14 of the '781 Patent.

J. O'Neill in view of De Luca, Olson, and Leung

The teachings of O'Neill, De Luca, Olson, and Leung are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of O'Neill, De Luca, Olson, and Leung render certain claims to be obvious. Thus, the technological teachings of O'Neill, De Luca, Olson, and Leung raise a substantial new question of patentability at least with respect to claims 5-13 of the '781 Patent.

K. Lapidus '178 in view of De Luca, Olson, and Hoepffner

The teachings of Lapidus '178, De Luca, and Olson are discussed briefly above.

Hoepffner is a journal article and discloses a comparison of characteristics of a new bedside immunological test strip device with a sensitive Guaiac-based and established immunochemical test for detection of faecal occult blood in patients. *Id.* at Abstract. The concentrations of hemoglobin in nanograms/milliliter (ng/ml) resulting in a positive blood test in fecal samples is discussed. Hoepffner was published and publicly available in 2006 and therefore is at least prior art under pre-AIA 35 U.S.C. § 102(b) against the challenged claims. Hoepffner was not cited during prosecution of the '781 Patent.

As discussed fully in Section VIII. below, the combined teachings of Lapidus '178, De Luca, Olson, and Hoepffner render certain claims to be obvious. Thus, the technological teachings of Lapidus '178, De Luca, Olson, and Hoepffner raise a substantial new question of patentability at least with respect to claims 16-20 of the '781 Patent.

L. Lapidus '650 in view of De Luca, Olson, and Hoepffner

Lapidus '650, De Luca, Olson, and Hoepffner are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Lapidus '650, De Luca, Olson, and Hoepffner render certain claims to be obvious. Thus, the technological teachings of Lapidus '650, De Luca, Olson, and Hoepffner raise a substantial new question of patentability at least with respect to claims 16-20 of the '781 Patent.

M. Ahlquist in view of De Luca, Olson, and Hoepffner

Ahlquist, De Luca, Olson, and Hoepffner are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of Ahlquist, De Luca, Olson, and Hoepffner render certain claims to be obvious. Thus, the technological teachings of Ahlquist, De Luca, Olson, and Hoepffner raise a substantial new question of patentability at least with respect to claims 16-20 of the '781 Patent.

N. O'Neill in view of De Luca, Olson, and Hoepffner

O'Neill, De Luca, Olson, and Hoepffner are discussed briefly above. As discussed fully in Section VIII. below, the combined teachings of O'Neill, De Luca, Olson, and Hoepffner render certain claims to be obvious. Thus, the technological teachings of O'Neill, De Luca, Olson, and Hoepffner raise a substantial new question of patentability at least with respect to claims 16-20 of the '781 Patent.

VII. Detailed Explanation under 37 C.F.R. § 1.510(b)

As discussed more fully below, certain challenged claims are anticipated by either Lapidus '178 or Lapidus '650 because each reference describes each and every element of the challenged claims. See, *e.g.,* Ex. 1014 at paragraphs 89 and 91. Specifically, Lapidus '178 and Lapidus '650 describe methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids. Fecal samples are collected from a human subject and were sealed in a container, separated into two or more portions, and combined with buffers.

In the alternative, certain challenged claims would have been obvious to one of ordinary skill in the art based on Lapidus '178 or Lapidus '650 because each reference discloses methods for processing and testing a fecal sample, including evaluation of proteins and nucleic acids as noted above. See, *e.g.,* Ex. 1014 at paragraphs 90 and 92.

As discussed more fully below, certain challenged claims would have been obvious to one of ordinary skill in the art, based on a combination of Lapidus '178/Lapidus '650, De Luca, and Olson, because the references disclose methods for processing and testing a fecal sample. See, *e.g.,* Ex. 1014 at paragraphs 93-94. Specifically, Lapidus '178 and Lapidus '650 disclose collection and separation of a fecal sample into containers and combining fecal samples with buffers. De Luca discloses combining a portion of a removed fecal sample and buffer in a

separate, sealable container for analyzing a blood protein such as hemoglobin. Olson discloses combining a portion of a fecal sample and buffer in a sealable container for analyzing human nucleic acid such as DNA.

A person of ordinary skill in the art would have been motivated to combine the teachings of Lapidus '178/Lapidus '650 with De Luca and Olsen to protect the fecal samples for improved capabilities of diagnostic tests in human patients. De Luca and Olsen specifically teach the advantages of separating fecal samples and stabilizing the fecal samples with buffers for improving diagnostic testing. See, *e.g.,* Ex. 1014 at paragraphs 65 and 83. Furthermore, modifying the methods of Lapidus '178/Lapidus '650 to include the features of De Luca and Olsen would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. *Id.*

A person of ordinary skill in the art would have been motivated by the express teachings of De Luca to modify the Lapidus '178/Lapidus '650 methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing a blood protein such as hemoglobin. *Id.* at paragraph 66. Specifically, De Luca discloses that its invention provides "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." Ex. 1006 at [0015].

Moreover, a person of ordinary skill in the art would also have been motivated by the express teachings of Olson to modify the Lapidus '178/Lapidus '650 methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing human nucleic acid such as DNA. See, *e.g.,* Ex. 1014 at paragraph 84. Olson explicitly sates that "[t]he goals of this investigation are to establish how sample handling conditions affect the stability of DNA in stool, thereby potentially limiting clinical sensitivity, and to determine conditions to ameliorate DNA degradation." Ex. 1007 at Abstract. Further, Olson concludes that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity." *Id.*

Accordingly, modifying the Lapidus '178/Lapidus '650 methods according to the advantages taught by De Luca and Olson would have been a "predictable use of prior art elements according to their established functions," yielding "predictable results." *KSR, supra,*

550 U.S. at 417. Because Lapidus '178/Lapidus '650 in combination with De Luca and Olson discloses all of the limitations of certain claims of the '781 Patent as explained herein, such claims would have been obvious to a person of ordinary skill in view of this prior art and unpatentable under 35 U.S.C. § 103. See, *e.g.,* Ex. 1014 at paragraphs 93-94.

As discussed more fully below, certain challenged claims would have been obvious to one of ordinary skill in the art, based on a combination of Ahlquist, De Luca, and Olson, because the references disclose methods for processing and testing a fecal sample. *Id.* at paragraph 95. Specifically, Ahlquist disclose collection and separation of a fecal sample into containers and combining fecal samples with buffers. De Luca discloses combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing a blood protein such as hemoglobin. Olson discloses combining a portion of a fecal sample and buffer in a sealable container for analyzing human nucleic acid such as DNA.

A person of ordinary skill in the art would have been motivated to combine the teachings of Ahlquist with De Luca and Olsen to protect the fecal samples for improved diagnostic power of cancer screening in human patients. De Luca and Olsen specifically teach the advantages of separating fecal samples and stabilizing the fecal samples with buffers for improving diagnostic testing. See, *e.g.,* Ex. 1014 at paragraphs 65 and 83. Furthermore, modifying the methods of Ahlquist include the features of De Luca and Olsen would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. *Id.* at paragraphs 68, 76, and 86.

A person of ordinary skill in the art would have been motivated by the express teachings of De Luca to modify the Ahlquist methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing a blood protein such as hemoglobin. *Id.* at paragraph 68. Specifically, De Luca discloses that its invention provides "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." Ex. 1006 at [0015].

Moreover, a person of ordinary skill in the art would also have been motivated by the express teachings of Olson to modify the Ahlquist methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing human nucleic acid such as DNA. Ex. 1014 at paragraph 86. Olson explicitly sates that "[t]he goals of this investigation

are to establish how sample handling conditions affect the stability of DNA in stool, thereby potentially limiting clinical sensitivity, and to determine conditions to ameliorate DNA degradation." Ex. 1007 at Abstract. Further, Olson concludes that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity." *Id.*

Accordingly, modifying the Ahlquist methods according to the advantages taught by De Luca and Olson would have been a "predictable use of prior art elements according to their established functions," yielding "predictable results." *KSR, supra,* 550 U.S. at 417. Because Ahlquist in combination with De Luca and Olson discloses all of the limitations of certain claims of the '781 Patent as explained herein, such claims would have been obvious to a person of ordinary skill in view of this prior art and unpatentable under 35 U.S.C. § 103. See, *e.g.,* Ex. 1014 at paragraph 95.

As discussed more fully below, certain challenged claims would have been obvious to one of ordinary skill in the art, based on a combination of O'Neill, De Luca, and Olson, because the references disclose methods for processing and testing a fecal sample. See, *e.g.,* Ex. 1014 at paragraph 96. Specifically, O'Neill disclose collection and separation of a fecal sample into containers and combining fecal samples with buffers. De Luca discloses combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing a blood protein such as hemoglobin. Olson discloses combining a portion of a fecal sample and buffer in a sealable container for analyzing human nucleic acid such as DNA.

A person of ordinary skill in the art would have been motivated to combine the teachings of O'Neill with De Luca and Olsen to protect the fecal samples for improved diagnostic power of cancer screening in human patients. De Luca and Olsen specifically teach the advantages of separating fecal samples and stabilizing the fecal samples with buffers for improving diagnostic testing. See, *e.g., id.* at paragraphs 65 and 83. Furthermore, modifying the methods of O'Neill include the features of De Luca and Olsen would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. *Id.* at paragraphs 70, 77, 87, and 88.

A person of ordinary skill in the art would have been motivated by the express teachings of De Luca to modify the O'Neill methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing a blood protein such as hemoglobin. *Id.*

at paragraph 70. Specifically, De Luca discloses that its invention provides "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." Ex. 1006 at [0015].

Moreover, a person of ordinary skill in the art would also have been motivated by the express teachings of Olson to modify the O'Neill methods by combining a portion of a removed fecal sample and buffer in a separate, sealable container for analyzing human nucleic acid such as DNA. Ex. 1014 at paragraphs 77 and 88. Olson explicitly sates that "[t]he goals of this investigation are to establish how sample handling conditions affect the stability of DNA in stool, thereby potentially limiting clinical sensitivity, and to determine conditions to ameliorate DNA degradation." Ex. 1007 at Abstract. Further, Olson concludes that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity." *Id.*

Accordingly, modifying the O'Neill methods according to the advantages taught by De Luca and Olson would have been a "predictable use of prior art elements according to their established functions," yielding "predictable results." *KSR, supra,* 550 U.S. at 417. Because O'Neill in combination with De Luca and Olson discloses all of the limitations of certain claims of the '781 Patent as explained herein, such claims would have been obvious to a person of ordinary skill in view of this prior art and unpatentable under 35 U.S.C. § 103. See, *e.g.,* Ex. 1014 at paragraph 96.

Moreover, Leung describes collection of stool and detection of fecal DNA and its analysis for hypermethylated DNA via PCR and for mRNA via RT-PCR. Ex. 1008 at Abstract. One skilled in the art would have been motivated to utilize the teachings Leung as additional testing that could have increased the predictive power of diagnosing colorectal cancer in human patients. *Id.* at paragraphs 171 and 191. Accordingly, modifying the teachings of i) Lapidus '178/Lapidus '650, De Luca, and Olson, ii) Ahlquist, De Luca, and Olson, and iii) O'Neill, De Luca, and Olson to incorporate the methods of Leung would have been a "predictable use of prior art elements according to their established functions," yielding "predictable results." *KSR*, supra, 550 U.S. at 417. Thus, because Leung in combination with i) Lapidus '178/Lapidus '650, De Luca, and Olson, ii) Ahlquist, De Luca, and Olson, and/or iii) O'Neill, De Luca, and Olson discloses all of the limitations of certain claims of the '781 Patent as explained herein, such claims would have been obvious to a person of ordinary skill in view of this prior art and unpatentable under 35 U.S.C. § 103. See, *e.g.,* Ex. 1014 at paragraphs 165 and 173.

Finally, Hoepffner describes concentrations of hemoglobin in nanograms per milliliter (ng/ml) resulting in a positive blood test in fecal samples. Ex. 1009 at p. 148, second column, first full paragraph. One skilled in the art would be motivated to utilize the teachings of Hoepffner in order to use the disclosed hemoglobin concentration as an indicator of a 'positive' test for blood in a fecal sample. Ex. 1014 at paragraph 219. Accordingly, modifying the teachings of i) Lapidus '178/Lapidus '650, De Luca, and Olson, ii) Ahlquist, De Luca, and Olson, and iii) O'Neill, De Luca, and Olson to incorporate the methods of Hoepffner would have been a "predictable use of prior art elements according to their established functions," yielding "predictable results." *KSR*, supra, 550 U.S. at 417. Thus, because Hoepffner in combination with i) Lapidus '178/Lapidus '650, De Luca, and Olson, ii) Ahlquist, De Luca, and Olson, and/or iii) O'Neill, De Luca, and Olson discloses all of the limitations of certain claims of the '781 Patent as explained herein, such claims would have been obvious to a person of ordinary skill in view of this prior art and unpatentable under 35 U.S.C. § 103. See, *e.g.,* Ex. 1014 at paragraphs 220-223.

A. Independent Claim 1

1. Lapidus '178 Anticipates Claim 1

'781 Patent, Claim 1, Preamble	Lapidus '178
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '178** describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

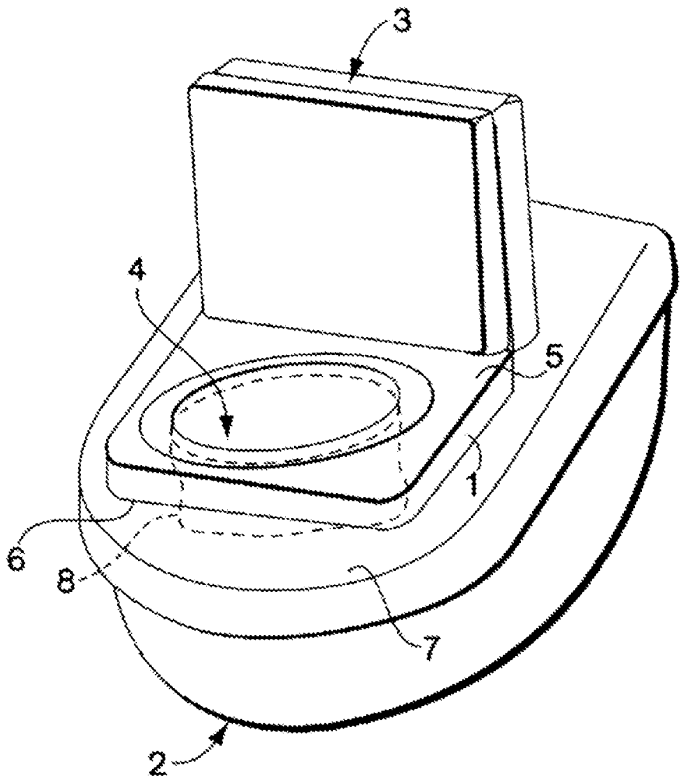
As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '178 describes this limitation. Lapidus '178 describes a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

Further, Lapidus '178 describes the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '178 describes that in "a preferred embodiment a stool is frozen prior to processing." *Id.* at column 9, lines 21-22. According to well-established law, the alternative (i.e., a stool NOT being frozen prior to processing) is also described by Lapidus '178. For instance, *In re Johnson* holds that if alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. See 558 F.2d 1008, 1019 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining."). See also *Ex parte Grasselli*, 231 USPQ 393 (Bd. App. 1983), *aff'd mem.*, 738 F.2d 453 (Fed. Cir. 1984). Accordingly, one skilled in the art would recognize Lapidus '178 describes the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Ex. 1014 at paragraph 45.

'781 Patent, Claim 1, First Limitation	**Lapidus '178**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**Lapidus '178** describes collecting "a stool voided by a patient." Ex. 1002 at column 7, lines 61-62. Lapidus '178 describes that a "[s]tool is voided [by the patient] into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64. Lapidus '178 describes that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. Lapidus '178 describes that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 8, lines 17-20.

Lapidus '178 describes collecting a fecal sample from a human subject. Lapidus '178 describes that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. This passage in Lapidus '178 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '178 describes that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 8, lines 24-27. Figure 2 of Lapidus '178 is shown below:



Finally, Lapidus '178 describes that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility. Lapidus '178 describes that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing

facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '178 describes that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '178 effectively describes collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '178 describes all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Second Limitation	**Lapidus '178**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**Lapidus '178** describes that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1002 at column 8, lines 45-47. Lapidus '178 describes that "[a]lternatively, a circumferential surface of a stool is prepared by taking the entire surface layer of the stool, or the entire surface layer of a cross-sectional portion of the stool." *Id.* at column 8, lines 49-52. Lapidus '178 describes that "[a]lternatively, the stool sample is rolled along the surface of the sampling device, and the layer of stool that adheres to the surface is then processed as described herein." *Id.* at column 9, lines 10-12.

	Referring to Figure 2, Lapidus '178 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 8, lines 33-37. Lapidus '178 describes that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 9, lines 30-32. Lapidus '178 describes "[o]nce a circumferential surface or cross-sectional sample of stool is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer." *Id.* at column 6, lines 45-56.

Lapidus '178 describes removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '178, the 'removed portion' could take a number of forms, including removal of a cross-sectional portion of the voided stool sample, a circumferential surface portion of the voided stool sample, or a layer of the voided stool sample that adheres to the surface of a sampling device. In these scenarios, the cross-sectional portion, the circumferential surface portion, or the layer portion described by Lapidus '178 corresponds to the 'removed portion' of the fecal sample in independent claim 1.

Given the removal of a cross-sectional, circumferential surface, and/or layer of the voided stool sample, logic dictates that Lapidus '178 describes a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '178 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

Moreover, Lapidus '178 describes that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '178 describes that "Means 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." This provides evidence that the 'removed portion' of the fecal sample (e.g., a circumferential surface or cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool. Ex. 1014 at paragraphs 60-63.

Finally, Lapidus '178 describes that the 'removed portion' of the fecal sample is in a separate sealable container. Lapidus '178 describes that the 'removed portion' (e.g., a circumferential surface or cross-sectional sample of stool) is placed in a buffer such as phosphate buffered saline according to "means and materials" that are generally known in the art in order to assay the 'removed portion'. At the time of filing Lapidus '178, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. For instance, U.S. Patent No. 4,859,610 to Maggio (Ex. 1010) describes a device that is "particularly useful for homogenizing and analyzing fecal samples." Ex. 1010 at column 4, lines 7-8. Maggio describes that the device includes a vessel for placement of a fecal sample and that "[a]fter a measured portion of [fecal sample] is secured

by the scoop (8) and introduced into the vessel (1), 2 mils of phosphate buffered saline, pH 7.4 (PBS) is added to the vessel (1)." *Id.* at column 8, lines 39-42.

Maggio later describes that "FIG. 1 is a perspective view of a preferred embodiment of the immunoassay incubation device." *Id.* at column 3, lines 33-34. Furthermore, Maggio describes that "FIG. 9 is an exploded view in perspective of another alternative embodiment of the immunoassay incubation device illustrating a valve for controlling the flow of homogenized sample from the extractor into the incubation chamber." *Id.* at column 3, lines 60-64. Figures 1 and 9 of Maggio, presented below, clearly depict a separate sealable container that is configured to contain a fecal sample and a homogenizing buffer:

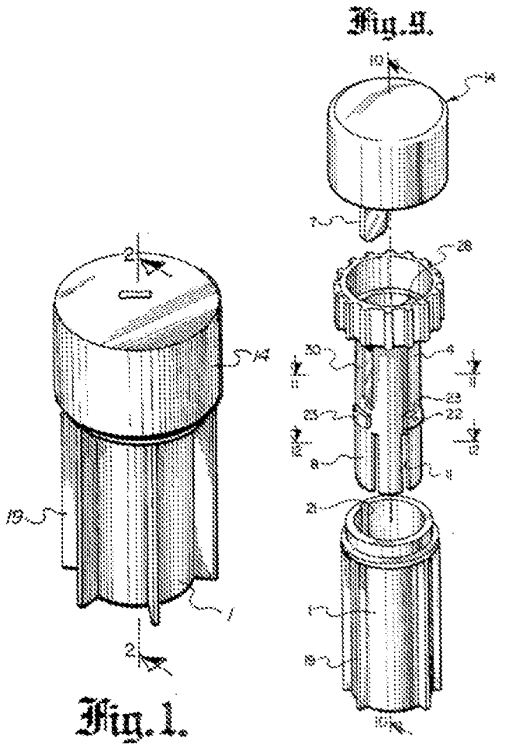


Fig. 1.

Therefore, one skilled in the art would recognize that Lapidus '178 describes all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 60-63.

'781 Patent, Claim 1, Third Limitation	Lapidus '178
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '178** describes that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1002 at column 9, lines 30-36.

Lapidus '178 describes combining the removed portion of the fecal sample (e.g., the cross-sectional portion, the circumferential surface portion, or the layer portion) with a buffer. Lapidus '178 describes such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at

paragraphs 72-73. Lapidus '178 further notes that to prevent the denaturation or degradation of blood proteins found in the sample, it would be important to specifically avoid including a proteinase in the buffer that would contain detergents and salts.

Again, Lapidus '178 describes that the cross-sectional portion, circumferential surface portion, or layer portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing. As discussed *supra,* at the time of filing Lapidus, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. See, *e.g.,* Maggio (Ex. 1010) as evidence of the state of the art at the time of filing Lapidus '178. Accordingly, the combination of the removed portion of the fecal sample and buffer is placed in a separate container and sealed according to methods known in the art.

Therefore, one skilled in the art would recognize that Lapidus '178 describes all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 73.

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '178**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '178** describes that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added). Lapidus '178 describes that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria

	present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The buffer may also contain inhibitors of DNA and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 8, lines 5-21.

Lapidus '178 describes combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. As discussed *supra*, given removal of a cross-sectional, circumferential surface, and/or layer of the stool, logic dictates that Lapidus '178 describes a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '178 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraph 79.

The 'remaining portion' of the fecal sample corresponds to the stool and Lapidus '178 describes that the stool is contained in the receptacle, which corresponds to the "sealable collection vessel." Further, Lapidus '178 describes that homogenization buffer can be added to the receptacle containing the stool. Lapidus '178 states that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase

inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added). This sentence is interpreted to describe two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. This sentence of Lapidus '178 describes addition of a buffer to the 'remaining portion' of ***the stool***.

Lapidus '178 describes that the buffer can contain phosphate buffered saline, NaCl or KCl, a detergent such as SDS or triton, and inhibitors of DNA and/or RNA degrading enzymes. A person of ordinary skill in the art would recognize such buffer as a "stabilizing buffer." Ex. 1014 at paragraph 80.

Finally, Lapidus '178 describes that the receptacle includes sealing means and that it should be sealed "to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors."

Therefore, one skilled in the art would recognize that Lapidus '178 describes all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 79-81.

2. Lapidus '650 Anticipates Claim 1

'781 Patent, Claim 1, Preamble	**Lapidus '650**
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '650** describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '650 describes this limitation. Lapidus '650 describes a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

Further, Lapidus '650 describes the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '650 does not describe the concept of freezing fecal samples. Thus, Lapidus explicitly describes collecting a fecal sample "without freezing" to meet the language of the preamble.

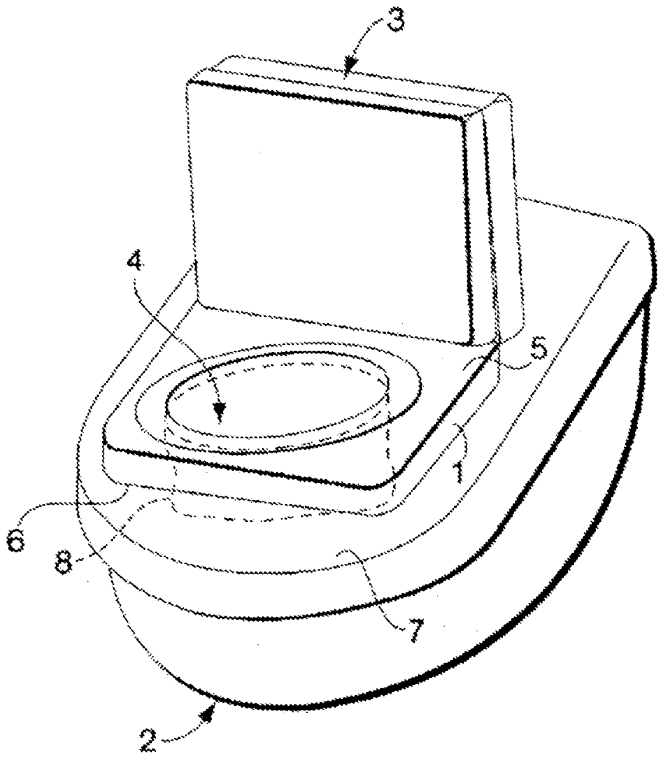
Moreover, U.S. Patent No. 5,196,167 (Ex. 1011) is incorporated by reference into Lapidus '650 at column 6, lines 30-31. In pertinent part, U.S. Patent No. 5,196,167 describes a test sheet product used for identification of blood in a patient stool and states that "[a]fter the patient has completed a bowel movement, the test sheet is merely dropped into the toilet bowl." Ex. 1011 at column 6, lines 65-67. Further, U.S. Patent No. 5,196,167 describes that "[a]fter the results of the test have been visually observed, the test sheet is disposed of by merely flushing the toilet bowl." *Id.* at column 7, lines 7-9. Accordingly, one skilled in the art would recognize Lapidus '650 describes the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble because U.S. Patent No. 5,196,167 (incorporated by reference into Lapidus '650) clearly describes a method in which a "freshly-collected" fecal sample is collected and processed "without freezing". Ex. 1014 at paragraph 46.

'781 Patent, Claim 1, First Limitation	**Lapidus '650**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**Lapidus '650** describes collecting "a stool voided by a patient." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26. Lapidus '650 describes that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 26-28. Lapidus '650 describes that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool

	sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 7, lines 45-49.

Lapidus '650 describes collecting a fecal sample from a human subject. Lapidus '650 describes that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 26-28. This passage in Lapidus '650 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '650 describes that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 7, lines 54-55. Figure 2 of Lapidus '650 is shown below:



Finally, Lapidus '650 describes that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility such a medical diagnostics laboratory. Lapidus '650 describes that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '650 describes that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '650 effectively describes collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '650 describes all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Second Limitation	**Lapidus '650**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**Lapidus '650** describes that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1003 at column 7, lines 17-19. Referring to Figure 2, Lapidus '650 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-

	sectional portion from the voided stool." *Id.* at column 7, lines 61-65. Lapidus '650 describes that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 8, lines 5-7. Lapidus '650 describes "[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer". *Id.* at column 6, lines 1-11.

Lapidus '650 describes removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '650, the cross-sectional portion corresponds to the 'removed portion' of the fecal sample in independent claim 1.

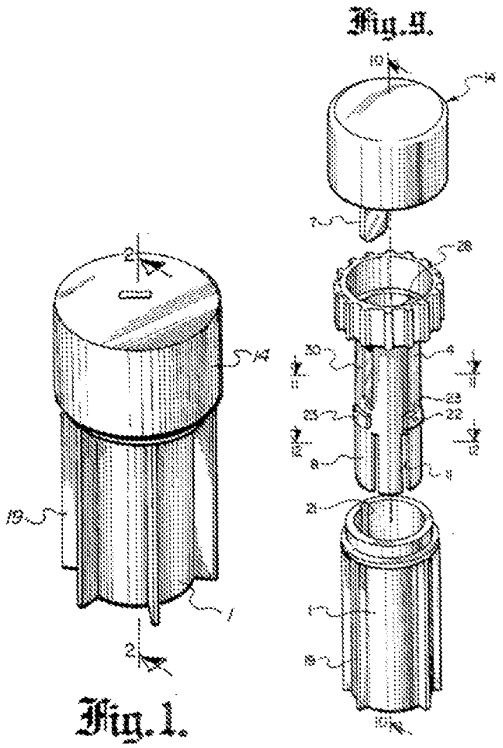
Given the removal of a cross-sectional sample, logic dictates that Lapidus '650 describes a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that

Lapidus '650 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

Moreover, Lapidus '650 describes that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '650 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 7, lines 61-65. This provides evidence that the 'removed portion' of the fecal sample (e.g., a cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

Finally, Lapidus '650 describes that the 'removed portion' of the fecal sample is in a separate sealable container. Lapidus '650 describes that the 'removed portion' (e.g., a cross-sectional sample of stool) is placed in a buffer such as phosphate buffered saline according to "means and materials" that are generally known in the art in order to assay the 'removed portion'. At the time of filing Lapidus '650, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. For instance, U.S. Patent No. 4,859,610 to Maggio (Ex. 1010) describes a device that is "particularly useful for homogenizing and analyzing fecal samples." Ex. 1010 at column 4, lines 7-8. Maggio describes that the device includes a vessel for placement of a fecal sample and that "[a]fter a measured portion of [fecal sample] is secured by the scoop (8) and introduced into the vessel (1), 2 mils of phosphate buffered saline, pH 7.4 (PBS) is added to the vessel (1)." *Id.* at column 8, lines 39-42.

Maggio later describes that "FIG. 1 is a perspective view of a preferred embodiment of the immunoassay incubation device." *Id.* at column 3, lines 33-34. Furthermore, Maggio describes that "FIG. 9 is an exploded view in perspective of another alternative embodiment of the immunoassay incubation device illustrating a valve for controlling the flow of homogenized sample from the extractor into the incubation chamber." *Id.* at column 3, lines 60-64. Figures 1 and 9 of Maggio, presented below, clearly depict a separate sealable container that is configured to contain a fecal sample and a homogenizing buffer:



Therefore, one skilled in the art would recognize that Lapidus '650 describes all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 60-63.

'781 Patent, Claim 1, Third Limitation	Lapidus '650
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '650** describes that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA

	and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1003 at column 8, lines 5-21.

Lapidus '650 describes combining the removed portion of the fecal sample (e.g., the cross-sectional portion) with a buffer. Lapidus '650 describes such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at paragraphs 72-73. Lapidus '650 further notes that to prevent the denaturation or degradation of blood proteins found in the sample, it would be important to specifically avoid including a proteinase in the buffer that would contain detergents and salts.

Again, Lapidus '650 describes that the cross-sectional portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing. As discussed *supra,* at the time of filing Lapidus, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. See, *e.g.,* Maggio (Ex. 1010) as evidence of the state of the art at the time of filing Lapidus '650. Accordingly, the combination of the removed portion of the fecal sample and buffer is placed in a separate container and sealed according to methods known in the art.

Therefore, one skilled in the art would recognize that Lapidus '650 describes all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 73.

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '650**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '650** describes that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1003 at column 2, line 66 – column 3, line 3 (emphasis added). Lapidus '650 describes that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The buffer may also contain inhibitors of DNA and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool

	sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 7, lines 31-49.

Lapidus '650 describes combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. As discussed *supra*, given removal of a cross-sectional sample of stool, logic dictates that Lapidus '650 describes a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraph 79.

The 'remaining portion' of the fecal sample corresponds to the stool and Lapidus '650 describes that the stool is contained in the receptacle, which corresponds to the "sealable collection vessel." Further, Lapidus '650 describes that homogenization buffer can be added to the receptacle containing the stool. Lapidus '650 states that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1003 at column 2, line 66 – column 3, line 3 (emphasis added). This sentence is interpreted to describe two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. This sentence of Lapidus '650 describes addition of a buffer to the 'remaining portion' of ***the stool***.

Lapidus '650 describes that the buffer can contain phosphate buffered saline, NaCl or KCl, a detergent such as SDS or triton, and inhibitors of DNA and/or RNA degrading enzymes. A person of ordinary skill in the art would recognize such buffer as a "stabilizing buffer." Ex. 1014 at paragraph 80.

Finally, Lapidus '650 describes that the receptacle includes sealing means and that it should be sealed "to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors."

Therefore, one skilled in the art would recognize that Lapidus '650 describes all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 79-81.

3. Lapidus '178 Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	Lapidus '178
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '178** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

In the alternative to the assertion that Lapidus '178 anticipates claim 1, Lapidus '178 further renders claim 1 obvious.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '178 discloses this limitation. Lapidus '178 discloses a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

Further, Lapidus '178 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '178 discloses that in "a preferred embodiment a stool is frozen prior to processing." *Id.* at column 9, lines 21-22. According to well-established law, the alternative (i.e., a stool NOT being frozen prior to processing) is also described by Lapidus. For instance, *In re Johnson* holds that if alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. See 558 F.2d 1008, 1019 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining."). See also *Ex parte Grasselli*, 231 USPQ 393 (Bd. App. 1983), *aff'd mem.*, 738 F.2d 453 (Fed. Cir. 1984). Accordingly, one skilled in the art would

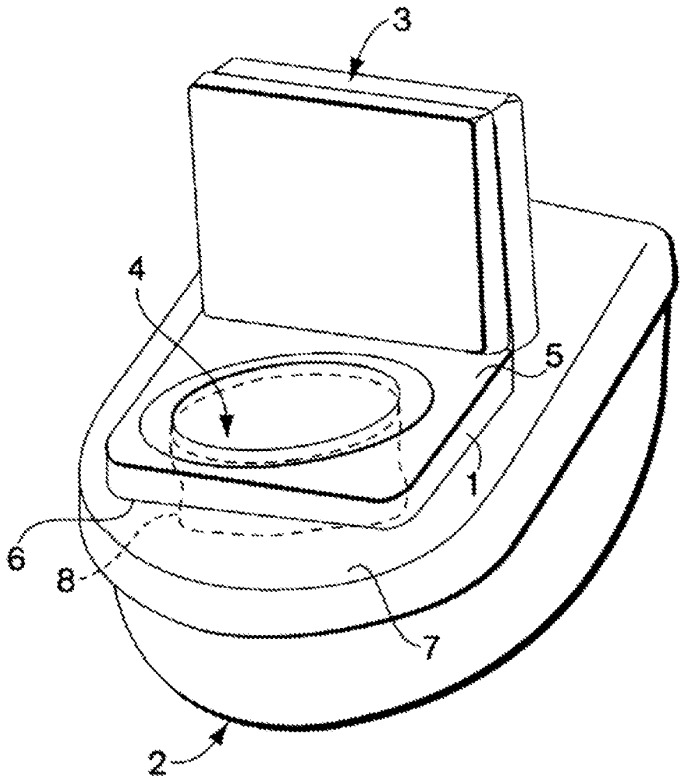
recognize Lapidus '178 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Ex. 1014 at paragraph 45.

'781 Patent, Claim 1, First Limitation	Lapidus '178
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**Lapidus '178** discloses collecting "a stool voided by a patient." Ex. 1002 at column 7, lines 61-62. Lapidus '178 discloses that a "[s]tool is voided [by the patient] into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64. Lapidus '178 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. Lapidus '178 discloses that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 8, lines 17-20.

Lapidus '178 discloses collecting a fecal sample from a human subject. Lapidus '178 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. This passage in Lapidus '178 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '178 discloses that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an

articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 8, lines 24-27. Figure 2 of Lapidus '178 is shown below:



Finally, Lapidus '178 discloses that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility. Lapidus '178 discloses that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '178 discloses that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '178 effectively describes collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.* Accordingly, Lapidus '178 renders this portion of claim 1 obvious.

'781 Patent, Claim 1, Second Limitation	**Lapidus '178**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**Lapidus '178** discloses that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1002 at column 8, lines 45-47. Lapidus '178 discloses that "[a]lternatively, a circumferential surface of a stool is prepared by taking the entire surface layer of the stool, or the entire surface layer of a cross-sectional portion of the stool." *Id.* at column 8, lines 49-52. Lapidus '178 discloses that "[a]lternatively, the stool sample is rolled along the surface of the sampling device, and the layer of stool that adheres to the surface is then processed as described herein." *Id.* at column 9, lines 10-12. Referring to Figure 2, Lapidus '178 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 8, lines 33-37.

	Lapidus '178 discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 9, lines 30-32. Lapidus '178 discloses "[o]nce a circumferential surface or cross-sectional sample of stool is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer." *Id.* at column 6, lines 45-56.

Lapidus '178 discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '178, the 'removed portion' could take a number of forms, including removal of a cross-sectional portion of the voided stool sample, a circumferential surface portion of the voided stool sample, or a layer of the voided stool sample that adheres to the surface of a sampling device. In these scenarios, the cross-sectional portion, the circumferential surface portion, or the layer portion described by Lapidus '178 corresponds to the 'removed portion' of the fecal sample in independent claim 1.

Given the removal of a cross-sectional, circumferential surface, and/or layer of the voided stool sample, it would be obvious that Lapidus '178 discloses a 'remaining portion' of the fecal sample that is left over following such removal. A person of ordinary skill in the art would recognize and understand that Lapidus '178 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

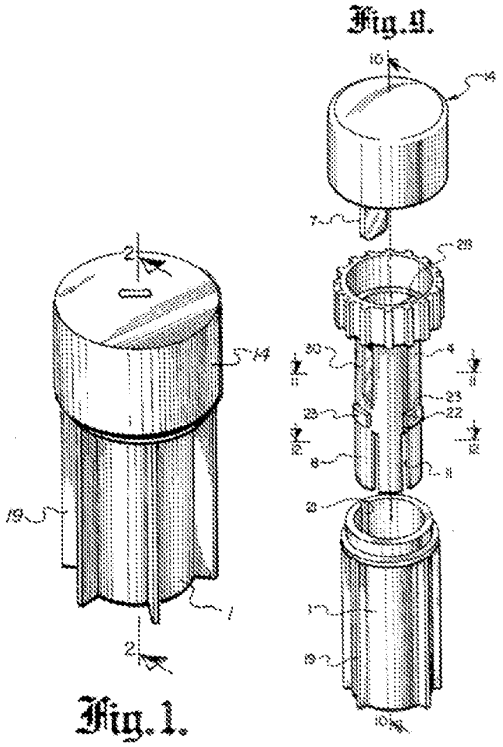
Moreover, Lapidus '178 discloses that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '178 discloses that "Means 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." This provides evidence that the 'removed portion' of the fecal sample (e.g., a circumferential surface or cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

Finally, Lapidus '178 discloses that the 'removed portion' of the fecal sample is in a separate sealable container. Lapidus '178 discloses that the 'removed portion' (e.g., a circumferential surface or cross-sectional sample of stool) is placed in a buffer such as phosphate buffered saline according to "means and materials" that are generally known in the art in order to assay the 'removed portion'. It would have been obvious that placement of the removed portion in a buffer would result in stabilization of the material in the portion for subsequent testing.

Further, at the time of filing Lapidus, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. For instance, U.S. Patent No. 4,859,610 to Maggio (Ex. 1010) describes a device that is "particularly useful for homogenizing and analyzing fecal samples." Ex. 1010 at column 4, lines 7-8. Maggio describes that the device includes a vessel for placement of a fecal sample and that "[a]fter a measured portion of [fecal sample] is secured by the scoop (8) and introduced into the vessel (1), 2 mils of phosphate buffered saline, pH 7.4 (PBS) is added to the vessel (1)." *Id.* at column 8, lines 39-42.

Maggio later describes that "FIG. 1 is a perspective view of a preferred embodiment of the immunoassay incubation device." *Id.* at column 3, lines 33-34. Furthermore, Maggio describes that "FIG. 9 is an exploded view in perspective of another alternative embodiment of the immunoassay incubation device illustrating a valve for controlling the flow of homogenized

sample from the extractor into the incubation chamber." *Id.* at column 3, lines 60-64. Figures 1 and 9 of Maggio, presented below, clearly depict a separate sealable container that is configured to contain a fecal sample and a homogenizing buffer:



Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 60-63. Accordingly, Lapidus '178 renders this portion of claim 1 obvious.

'781 Patent, Claim 1, Third Limitation	**Lapidus '178**
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '178** discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally

	known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1002 at column 9, lines 30-36.

Lapidus '178 discloses combining the removed portion of the fecal sample (e.g., the cross-sectional portion, the circumferential surface portion, or the layer portion) with a buffer. Lapidus '178 discloses such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at paragraphs 72-73.

Again, Lapidus '178 discloses that the cross-sectional portion, circumferential surface portion, or layer portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing. As discussed *supra,* at the time of filing Lapidus, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. See, *e.g.,* Maggio (Ex. 1010) as

evidence of the state of the art at the time of filing Lapidus. Accordingly, the combination of the removed portion of the fecal sample and buffer is placed in a separate container and sealed according to methods known in the art.

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 73. Accordingly, Lapidus '178 renders this portion of claim 1 obvious.

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '178**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '178** discloses that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added). Lapidus '178 discloses that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The

	buffer may also contain inhibitors of DNA and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 8, lines 5-21.

Lapidus '178 discloses combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. As discussed *supra*, given removal of a cross-sectional, circumferential surface, and/or layer of the stool, logic dictates that Lapidus '178 discloses a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '178 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraph 79.

The 'remaining portion' of the fecal sample corresponds to the stool and Lapidus '178 discloses that the stool is contained in the receptacle, which corresponds to the "sealable collection vessel." Further, Lapidus '178 discloses that homogenization buffer can be added to the receptacle containing the stool. Lapidus '178 states that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added). This sentence is interpreted ti describe two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. This sentence of Lapidus '178 discloses addition of a buffer to the 'remaining portion' of ***the stool***.

Lapidus '178 discloses that the buffer can contain phosphate buffered saline, NaCl or KCl, a detergent such as SDS or triton, and inhibitors of DNA and/or RNA degrading enzymes. A person of ordinary skill in the art would recognize such buffer as a "stabilizing buffer." Ex. 1014 at paragraph 80.

Finally, Lapidus '178 discloses that the receptacle includes sealing means and that it should be sealed "to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors."

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 79-81. Accordingly, Lapidus '178 renders this portion of claim 1 obvious.

4. Lapidus '650 Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	Lapidus '650
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '650** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

In the alternative to the assertion that Lapidus '650 anticipates claim 1, Lapidus '650 further renders claim 1 obvious.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '650 discloses this limitation. Lapidus '650 discloses a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

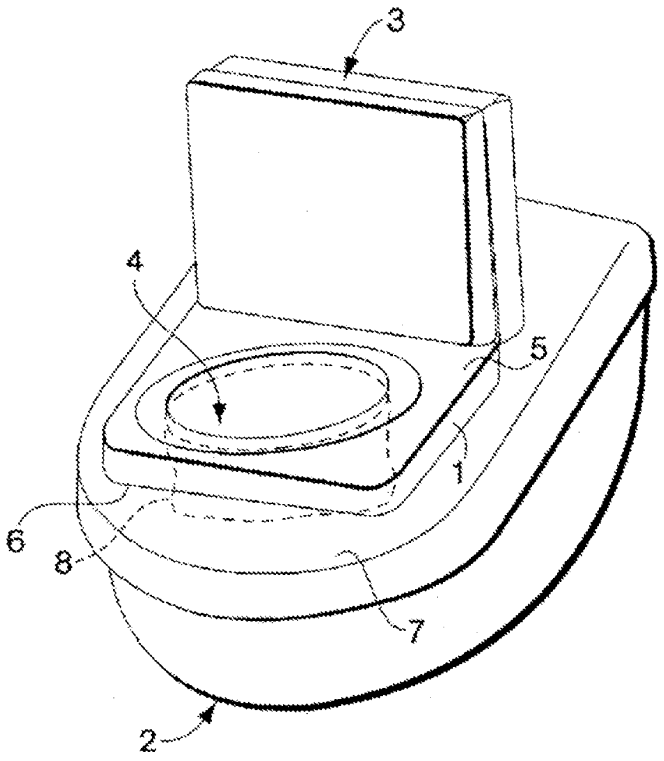
Further, Lapidus '650 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '650 does not disclose the concept of freezing fecal samples. Thus, Lapidus explicitly discloses collecting a fecal sample "without freezing" to meet the language of the preamble.

Moreover, U.S. Patent No. 5,196,167 (Ex. 1011) is incorporated by reference into Lapidus '650 at column 6, lines 30-31. In pertinent part, U.S. Patent No. 5,196,167 discloses a test sheet product used for identification of blood in a patient stool and states that "[a]fter the patient has completed a bowel movement, the test sheet is merely dropped into the toilet bowl." Ex. 1011 at column 6, lines 65-67. Further, U.S. Patent No. 5,196,167 discloses that "[a]fter the results of the test have been visually observed, the test sheet is disposed of by merely flushing the toilet bowl." *Id.* at column 7, lines 7-9. Accordingly, one skilled in the art would recognize Lapidus '650 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble because U.S. Patent No. 5,196,167 (incorporated by reference into Lapidus '650) clearly discloses a method in which a "freshly-collected" fecal sample is collected and processed "without freezing". Ex. 1014 at paragraph 45.

'781 Patent, Claim 1, First Limitation	**Lapidus '650**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**Lapidus '650** discloses collecting "a stool voided by a patient." Ex. 1003 at column 7, lines 61-62. Lapidus '650 discloses that a "[s]tool is voided [by the patient] into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64. Lapidus '650 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. Lapidus '650 discloses that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 8, lines 17-20.

Lapidus '650 discloses collecting a fecal sample from a human subject. Lapidus '650 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 26-28. This passage in Lapidus '650 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '650 discloses that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 7, lines 54-55. Figure 2 of Lapidus '650 is shown below:



Finally, Lapidus '650 discloses that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility such a medical diagnostics laboratory. Lapidus

'650 discloses that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '650 discloses that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '650 effectively discloses collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.* Accordingly, Lapidus '650 renders this portion of claim 1 obvious.

a.

'781 Patent, Claim 1, Second Limitation	**Lapidus '650**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**Lapidus '650** discloses that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1003 at column 8, lines 45-47. Lapidus '650 discloses that "[a]lternatively, a circumferential surface of a stool is prepared by taking the entire surface layer of the stool, or the entire surface layer of a cross-sectional portion of the stool." *Id.* at column 8, lines 49-52. Lapidus '650 discloses that "[a]lternatively, the stool sample is rolled along the surface of the sampling device, and the layer of stool that adheres to the surface is then processed as described herein." *Id.* at column 9, lines 10-12.

	Referring to Figure 2, Lapidus '650 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 8, lines 33-37. Lapidus '650 discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 9, lines 30-32. Lapidus '650 discloses "[o]nce a circumferential surface or cross-sectional sample of stool is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics

	indicative of colorectal cancer. *Id.* at column 6, lines 45-56.

Lapidus '650 discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '650, the cross-sectional portion corresponds to the 'removed portion' of the fecal sample in independent claim 1.

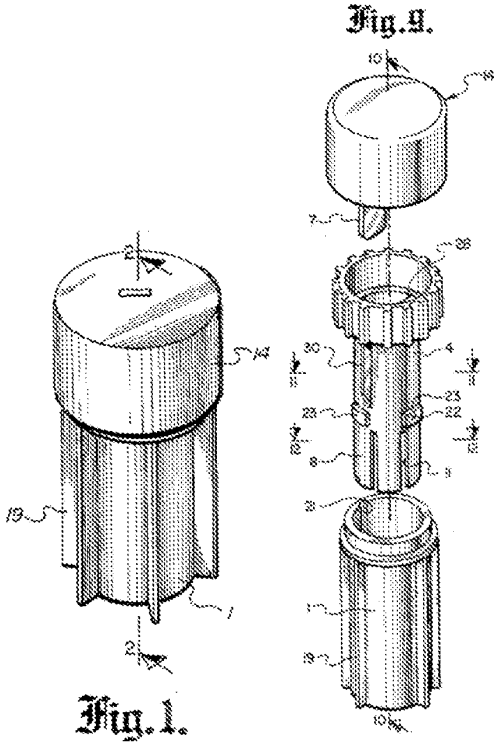
Given the removal of a cross-sectional sample, logic dictates that Lapidus '650 discloses a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

Moreover, Lapidus '650 discloses that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '650 discloses that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 7, lines 61-65. This provides evidence that the 'removed portion' of the fecal sample (e.g., a cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

Finally, Lapidus '650 discloses that the 'removed portion' of the fecal sample is in a separate sealable container. Lapidus '650 discloses that the 'removed portion' (e.g., a cross-sectional sample of stool) is placed in a buffer such as phosphate buffered saline according to "means and materials" that are generally known in the art in order to assay the 'removed portion'. At the time of filing Lapidus '650, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. For instance, U.S. Patent No. 4,859,610 to Maggio (Ex. 1010) discloses a device that is "particularly useful for homogenizing and analyzing fecal samples." Ex. 1010 at column 4, lines 7-8. Maggio discloses that the device includes a vessel for placement of a fecal sample and that "[a]fter a measured portion of [fecal sample] is secured by the scoop (8) and

introduced into the vessel (1), 2 mils of phosphate buffered saline, pH 7.4 (PBS) is added to the vessel (1)." *Id.* at column 8, lines 39-42.

Maggio later discloses that "FIG. 1 is a perspective view of a preferred embodiment of the immunoassay incubation device." *Id.* at column 3, lines 33-34. Furthermore, Maggio discloses that "FIG. 9 is an exploded view in perspective of another alternative embodiment of the immunoassay incubation device illustrating a valve for controlling the flow of homogenized sample from the extractor into the incubation chamber." *Id.* at column 3, lines 60-64. Figures 1 and 9 of Maggio, presented below, clearly depict a separate sealable container that is configured to contain a fecal sample and a homogenizing buffer:



Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 60-63. Accordingly, Lapidus '650 renders this portion of claim 1 obvious.

'781 Patent, Claim 1, Third Limitation	Lapidus '650
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '650** discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1003 at column 9, lines 30-36.

Lapidus '650 discloses combining the removed portion of the fecal sample (e.g., the cross-sectional portion) with a buffer. Lapidus '650 discloses such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at paragraphs 72-73. Lapidus '650 further notes that to

prevent the denaturation or degradation of blood proteins found in the sample, it would be important to specifically avoid including a proteinase in the buffer that would contain detergents and salts.

Again, Lapidus '650 discloses that the cross-sectional portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing. As discussed *supra,* at the time of filing Lapidus, it was well known in the art that a fecal sample was homogenized in appropriate buffers for the purpose of assaying would be done in a separate sealable container. See, *e.g.,* Maggio (Ex. 1010) as evidence of the state of the art at the time of filing Lapidus '650. Accordingly, the combination of the removed portion of the fecal sample and buffer is placed in a separate container and sealed according to methods known in the art.

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 73. Accordingly, Lapidus '650 renders this portion of claim 1 obvious.

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '650**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '650** discloses that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1003 at column 3, lines 11-15 (emphasis added). Lapidus '650 discloses that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria

	present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The buffer may also contain inhibitors of DNA and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 8, lines 5-21.

Lapidus '650 discloses combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. As discussed *supra*, given removal of a cross-sectional sample of stool, logic dictates that Lapidus '650 discloses a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraph 79.

The 'remaining portion' of the fecal sample corresponds to the stool and Lapidus '650 discloses that the stool is contained in the receptacle, which corresponds to the "sealable collection vessel." Further, Lapidus '650 discloses that homogenization buffer can be added to the receptacle containing the stool. Lapidus '650 states that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1003 at column 2, line 66 – column 3, line 3 (emphasis added). This sentence is

interpreted to disclose two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. This sentence of Lapidus '650 discloses addition of a buffer to the 'remaining portion' of ***the stool***.

Lapidus '650 discloses that the buffer can contain phosphate buffered saline, NaCl or KCl, a detergent such as SDS or triton, and inhibitors of DNA and/or RNA degrading enzymes. A person of ordinary skill in the art would recognize such buffer as a "stabilizing buffer." Ex. 1014 at paragraph 80.

Finally, Lapidus '650 discloses that the receptacle includes sealing means and that it should be sealed "to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors."

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 79-81. Accordingly, Lapidus '650 renders this portion of claim 1 obvious.

5. Lapidus '178 in view of De Luca and Olson Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	Lapidus '178 in view of De Luca and Olson
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '178** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '178 discloses this limitation. Lapidus '178 discloses a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

Further, Lapidus '178 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '178 discloses that in "a preferred embodiment a stool is frozen prior to processing." *Id.* at column 9, lines 21-22. According to well-established law, the alternative (i.e., a stool NOT being frozen prior to processing) is also described by Lapidus. For instance, *In re Johnson* holds that if alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. See 558 F.2d 1008, 1019 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining."). See also *Ex parte Grasselli*, 231 USPQ 393 (Bd. App. 1983), *aff'd mem.*, 738 F.2d 453 (Fed. Cir. 1984). Accordingly, one skilled in the art would recognize Lapidus '178 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Ex. 1014 at paragraph 45.

'781 Patent, Claim 1, First Limitation	**Lapidus '178 in view of De Luca and Olson**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**Lapidus '178** discloses collecting "a stool voided by a patient." Ex. 1002 at column 7, lines 61-62. Lapidus '178 discloses that a "[s]tool is voided [by the patient] into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64. Lapidus '178 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. Lapidus '178 discloses that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 8, lines 17-20.

Lapidus '178 discloses collecting a fecal sample from a human subject. Lapidus '178 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 64-66. This passage in Lapidus '178 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '178 discloses that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 8, lines 24-27. Figure 2 of Lapidus '178 is shown *supra.*

Finally, Lapidus '178 discloses that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility. Lapidus '178 discloses that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '178 discloses that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '178 effectively describes collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Second Limitation	**Lapidus '178 in view of De Luca and Olson**
b) removing a portion of the fecal sample to a separate sealable container to produce a	**Lapidus '178** discloses that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal

removed portion and a remaining portion of the fecal sample;	section through the stool." Ex. 1002 at column 8, lines 45-47. Lapidus '178 discloses that "[a]lternatively, a circumferential surface of a stool is prepared by taking the entire surface layer of the stool, or the entire surface layer of a cross-sectional portion of the stool." *Id.* at column 8, lines 49-52. Lapidus '178 discloses that "[a]lternatively, the stool sample is rolled along the surface of the sampling device, and the layer of stool that adheres to the surface is then processed as described herein." *Id.* at column 9, lines 10-12. Referring to Figure 2, Lapidus '178 describes that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 8, lines 33-37. Lapidus '178 discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 9, lines 30-32. Lapidus '178 discloses "[o]nce a circumferential surface or cross-sectional sample of stool is obtained, it may be

	homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer." *Id.* at column 6, lines 45-56. **De Luca** discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces

	samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

Lapidus '178 discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '178, the 'removed portion' could take a number of forms, including removal of a cross-sectional portion of the voided stool sample, a circumferential surface portion of the voided stool sample, or a layer of the voided stool sample that adheres to the surface of a sampling device. In these scenarios, the cross-sectional portion, the circumferential surface portion, or the layer portion described by Lapidus '178 corresponds to the 'removed portion' of the fecal sample in independent claim 1.

Given the removal of a cross-sectional, circumferential surface, and/or layer of the voided stool sample, it would be obvious that Lapidus '178 discloses a 'remaining portion' of the fecal sample that is left over following such removal. A person of ordinary skill in the art would recognize and understand that Lapidus '178 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

Moreover, Lapidus '178 discloses that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '178 discloses that "Means 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." This provides evidence that the 'removed portion' of the fecal sample (e.g., a circumferential surface or cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

Even if Lapidus '178 is determined to not specifically teach removal to a separate sealable container, De Luca removes this deficiency. De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform assays, such as fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a fecal sample with buffer solution and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the 'removed portion' of the fecal sample obtained by Lapidus '178 with the requisite expectation of success. Therefore, one skilled in the art would recognize that Lapidus '178 in view of De Luca discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 66.

'781 Patent, Claim 1, Third Limitation	**Lapidus '178 in view of De Luca and Olson**
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '178** discloses that "[o]nce obtained, the entire circumference or cross-sectional sample of stool is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the

	buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1002 at column 9, lines 30-36. **De Luca** discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably

	to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

Lapidus '178 discloses combining the removed portion of the fecal sample (e.g., the cross-sectional portion, the circumferential surface portion, or the layer portion) with a buffer. Lapidus '178 discloses such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at paragraphs 72-73.

Again, Lapidus '178 discloses that the cross-sectional portion, circumferential surface portion, or layer portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing.

Even if Lapidus '178 is determined to not specifically teach combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container, De Luca removes this deficiency. De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are used for detection of blood proteins such as hemoglobin for use in fecal immunochemical blood tests. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a buffer solution and fecal sample and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the removed portion from the fecal sample of Lapidus '178. Based on De Luca, a person of ordinary skill in the art would combine the removed portion of the fecal sample in the separate sealable container with a buffer in the

extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Ex. 1014 at paragraph 75. Further, De Luca teaches that the extraction tube is sealed using a top cap, rendering the extraction tube to be a "separate sealable container." Therefore, one skilled in the art would recognize that Lapidus '178 in view of De Luca discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '178 in view of De Luca and Olson**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '178** discloses that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added). Lapidus '178 discloses that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The buffer may also contain inhibitors of DNA

	and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 8, lines 5-21. **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with

	stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '178 discloses combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. Even if Lapidus '178 is determined to not specifically teach combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, Olson removes this deficiency.

Olson discloses that addition of a stabilizing buffer (Exact buffer A) to stool samples provided a significant increase in recovery of DNA compared with samples incubated without buffer. This addition of a stabilizing buffer to DNA led to increased assay sensitivity.

Thus, a person of ordinary skill in the art would have been motivated to combine the remaining portion of the fecal sample with the stabilizing buffer taught by Olson. Olson discloses that DNA assays are greatly improved with addition of stabilizing buffer. Therefore, one skilled in the art would recognize that Lapidus '178 in view of Olson discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 84.

In summary, Lapidus '178 in view of De Luca and Olson disclose the limitations of claim 1 of the '781 Patent. Consequently, claim 1 is obvious over Lapidus '178 in view of De Luca and Olson.

6. Lapidus '650 in view of De Luca and Olson Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	**Lapidus '650 in view of De Luca and Olson**
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**Lapidus '650** describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at

	column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Lapidus '650 discloses this limitation. Lapidus '650 discloses a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility.

Further, Lapidus '650 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Lapidus '650 does not disclose the concept of freezing fecal samples. Thus, Lapidus explicitly discloses collecting a fecal sample "without freezing" to meet the language of the preamble.

Moreover, U.S. Patent No. 5,196,167 (Ex. 1011) is incorporated by reference into Lapidus '650 at column 6, lines 30-31. In pertinent part, U.S. Patent No. 5,196,167 discloses a test sheet product used for identification of blood in a patient stool and states that "[a]fter the patient has completed a bowel movement, the test sheet is merely dropped into the toilet bowl." Ex. 1011 at column 6, lines 65-67. Further, U.S. Patent No. 5,196,167 discloses that "[a]fter the results of the test have been visually observed, the test sheet is disposed of by merely flushing the toilet bowl." *Id.* at column 7, lines 7-9. Accordingly, one skilled in the art would recognize Lapidus '650 discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble because U.S. Patent No. 5,196,167 (incorporated by reference into Lapidus '650) clearly discloses a method in which a "freshly-collected" fecal sample is collected and processed "without freezing". Ex. 1014 at paragraph 45.

'781 Patent, Claim 1, First Limitation	**Lapidus '650 in view of De Luca and Olson**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected	**Lapidus '650** discloses collecting "a stool voided by a patient." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a

at home by the human subject by defecation directly into a sealable collection vessel;	receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26. Lapidus '650 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 26-28. Lapidus '650 discloses that "[t]he receptacle, whether adapted to fit a toilet or simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample…". *Id.* at column 7, lines 45-49.

Lapidus '650 discloses collecting a fecal sample from a human subject. Lapidus '650 discloses that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." *Id.* at column 7, lines 26-28. This passage in Lapidus '650 provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

Furthermore, Lapidus '650 discloses that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2, the receptacle is a "sealable" receptacle comprising an articulating **cover 3** that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." *Id.* at column 7, lines 54-55. Figure 2 of Lapidus '650 is shown *supra.*

Finally, Lapidus '650 discloses that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. As discussed above, the broadest reasonable interpretation of "collected at home" in the '781 Patent is collected at a location other than a testing facility such a medical diagnostics laboratory. Lapidus '650 discloses that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility," meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '650 discloses that the toilet onto

which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." This indicates that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. A person skilled in the art would understand that Lapidus '650 effectively discloses collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory. Ex. 1014 at paragraph 53.

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Second Limitation	**Lapidus '650 in view of De Luca and Olson**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**Lapidus '650** discloses that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1003 at column 7, lines 17-19. Referring to Figure 2, Lapidus '650 discloses that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 7, lines 61-65. Lapidus '650 discloses that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 8, lines 5-7. Lapidus '650 discloses "[o]nce a

	cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer". *Id.* at column 6, lines 1-11. **De Luca** discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for