

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GENEOSCOPY, INC.,
Petitioner,

v.

EXACT SCIENCES CORPORATION,
Patent Owner.

Case No.: IPR2024-00459
U.S. Patent No. 11,634,781

PETITIONER'S DEMONSTRATIVES

Pursuant to the Board's Order Setting Oral Argument dated March 19, 2025,
Petitioner Geneoscopy, Inc.'s demonstratives appended to this filing.

Dated: April 28, 2025

Respectfully submitted,

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CERTIFICATE OF SERVICE

The undersigned hereby certifies that a true and correct copy of the foregoing **PETITIONER'S DEMONSTRATIVES** was served on April 28, 2025 via the delivery method indicated to the attorneys of record for the Patent Owner as follows:

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Dated: April 28, 2025

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ORAL ARGUMENT FOR IPR2024-00459

U.S. Patent No. 11,634,781

Petitioner's Hearing Demonstratives

May 1, 2025

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Introduction

Petition, at pp. 9-11; EX1001

Demonstrative Exhibit - Not Evidence

[illegible]

(10) **Patent No.:** US 11,634,781 B2
(45) **Date of Patent:** *Apr. 25, 2023

(54) **FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**

(71) Applicant: **Exact Sciences Corporation**, Madison, WI (US)

(72) Inventor: **Joost Louwagie**, Dornach (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 17/936,335

(22) Filed: Sep. 28, 2022

(65) **Prior Publication Data**
US 2023/0048152 A1 Feb. 16, 2023

Related U.S. Application Data

(63) Continuation of application No. 15/634,607, filed on Jun. 27, 2017, which is a continuation of application No. 15/010,436, filed on Jan. 29, 2016, now abandoned, which is a continuation of application No. 13/147,570, filed as application No. PCT/GB2010/000180 on Feb. 3, 2010, now abandoned.

(60) Provisional application No. 61/149,581, filed on Feb. 3, 2009.

Claims of the '781 Patent Are Directed to Performance of Two Prior Art Assays to Detect CRC in a Fecal Sample

US011634781B2

**(12) United States Patent
Louwage**

(10) Patent No.: US 11,634,781 B2
(45) Date of Patent: Apr. 25, 2023

(54) **FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**

(71) Applicant: **Exact Sciences Corporation**, Madison, WI (US)

(72) Inventor: **Joost Louwage**, Dornach (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days. This patent is subject to a terminal disclaimer.

(21) Appl. No.: **179796,335**

(22) Filed: **Sep. 28, 2022**

(65) **Publication Data**
US 2023/0048152 A1 Feb. 16, 2023

Related U.S. Application Data

(63) Continuation of application No. 15/634,607, filed on Jan. 27, 2017, which is a continuation of application No. 15/610,436, filed on Jan. 29, 2016, now abandoned, which is a continuation of application No. 13/147,570, filed as application No. PCT/CH2010/000180 on Feb. 3, 2010, now abandoned.

(66) Provisional application No. 61/149,581, filed on Feb. 3, 2009.

(Int. Cl.)
C12Q 1/6886 (2018.01)
WO/2009/18374 (2009.01)

(U.S. Cl.)
CPC — **C12Q 1/6886** (2013.01); **GIN 33/57446** (2013.01); **C12Q 2600/158** (2013.01); **C12Q 2600/153** (2013.01); **GIN 2233/805** (2013.01); **GIN 2400/20** (2013.01); **GIN 2400/22** (2013.01)

(58) **Field of Classification Search**
CPC — **C12Q 1/68**; **C12Q 2600/154**; **C12Q 2600/158**; **GIN 33/57446**; **GIN 2233/805**

See application file for complete search history.

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(57)

A method of processing fecal sample comprising removing and adding the reagent prevents denaturation in the sample, and the removed particles comprise stabilizing

Specific

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.

3. A method of processing a fecal sample, the method comprising:
- a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and
 - ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,the pair of portions obtained by the method of claim 1;
 - b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;
 - c) extracting nucleic acid from the remaining portion of the fecal sample; and
 - d) testing the nucleic acid for an amount of a human nucleic acid.

Petition, at pp. 1, 25-26, 50-51; EX1001

Demonstrative Exhibit - Not Evidence

State of the Art: Immunochemical Fecal Occult Blood Tests (iFOBT or FIT)

1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:

- 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

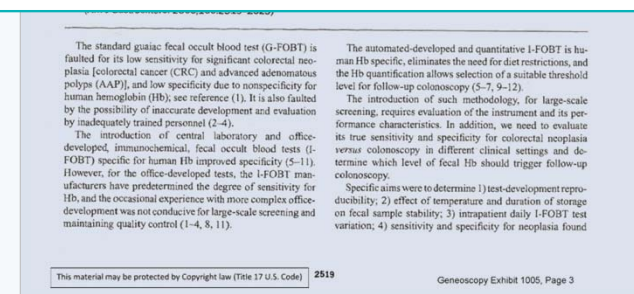
3. A method of processing a fecal sample, the method comprising:

- a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and

- b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;



Steps of at-home iFOBT assay, as disclosed in Vilkin (EX1005)



Petition, at pp. 1, 25-26, 50-51; EX1005 (Vilkin)

Demonstrative Exhibit - Not Evidence

State of the Art: Fecal DNA CRC Assays

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;

d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:

ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Improved Fecal DNA Test for Colorectal Cancer Screening

STEVEN H. ITZKOWITZ,* LINA JANDORF,* RANDALL BRAND,* LINDA RABENECK,* PAUL C. SCHROY III,[†] STEPHEN SONTAG,* DAVID JOHNSON,* JOEL SKOLETNIK,* KRIS DUNKEE,* SANFORD MARKOWITZ,[‡] AND ANTHONY SHUBER*

*Department of Medicine and Oncological Sciences, Mount Sinai School of Medicine, New York, New York; [†]Gastroenterology Division, Evanston Northwestern Healthcare, Evanston, Illinois; [‡]Florida Gastrointestinal Regional Cancer Center, University School of Medicine, Boston, Massachusetts; [§]Gastroenterology and Hepatology Division, University of California, San Francisco, California; ^{||}Medical Oncology, Cleveland Clinic Foundation, Cleveland, Ohio

Background & Aims: Fecal DNA testing has also greater sensitivity than guaiac-based occult blood test for noninvasive colorectal cancer (CRC) screening. A prototype assay (version 1), which analyzed 22 gene mutations and DNA integrity assay (DIA), showed a sensitivity of 82% for CRC detection and a specificity of 94% in at-risk individuals. The present study was conducted

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau

(43) International Publication Date 1 December 2005 (01.12.2005)



(10) International Publication Number WO 2005/113769 A1

Steps of at-home fecal DNA assay, as disclosed in Itzkowitz (EX1006) and Shuber (EX1009)

availability of an accurate, noninvasive screening test may increase compliance with CRC screening guidelines by individuals who are reluctant to undergo more invasive tests, or situations in which colonoscopy screening is not feasible or not available.

Several studies have shown the feasibility of detecting colorectal cancer-specific products in the stool. The markers in this study represent alterations of genes involved in the protein-coding chromosomal instability pathway (such as APC, p53, c

WO 2005/113769 A1

(54) Title: METHOD FOR STABILIZING BIOLOGICAL SAMPLES FOR NUCLEIC ACID ANALYSIS

(57) Abstract: Disclosed is a method for preparing nucleic acid containing biological samples for a nucleic acid integrity assay and/or multiple mutation analysis by incubating the biological sample with a stabilization solution that includes a buffer, a chelating agent and a salt.

Petition, at pp. 1, 25-26, 50-51; EX1006 (Itzkowitz); EX1009 (Shuber)

Demonstrative Exhibit - Not Evidence

'781 Patent Claims Are Directed to Performance of Two Prior Art Assays to Detect CRC in a Fecal Sample

	
US 11,634,781 B2	
(12) United States Patent Louwagie	(10) Patent No.: US 11,634,781 B2 (45) Date of Patent: *Apr. 25, 2023
(54) FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD (71) Applicant: Exact Sciences Corporation, Madison, WI (US) (72) Inventor: Jost Louwagie, Domach (CH) (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(d) by 0 days. This patent is subject to a terminal disclaimer. (21) Appl. No.: 17/936,335 (22) Filed: Sep. 28, 2022 (65) Prior Publication Data US 2023/0048152 A1 Feb. 16, 2023 Related U.S. Application Data (63) Continuation of application No. 15/034,607, filed on Jun. 27, 2017, which is a continuation of application No. 15/010,436, filed on Jan. 29, 2016, now abandoned, which is a continuation of application No. 13/147,570, filed as application No. PCT/US2010/000180 on Feb. 3, 2010, now abandoned. (69) Provisional application No. 61/149,581, filed on Feb. 3, 2009. (51) Int. Cl. C12Q 1/6886 (2018.01) G01N 33/574 (2006.01) (52) U.S. Cl. CPC — C12Q 1/6886 (2013.01); G01N 33/57446 (2013.01); C12Q 2600/156 (2013.01); C12Q 2600/152 (2013.01); C12Q 2600/158 (2013.01); G01N 2333/805 (2013.01); G01N 2600/50 (2013.01); G01N 2600/52 (2013.01) (58) Field of Classification Search CPC — C12Q 1/68; C12Q 2600/154; C12Q 2600/158; G01N 33/57446; G01N 2333/805 See application file for complete search history. (56) References Cited U.S. PATENT DOCUMENTS 4,447,975 A 5/1984 Gillette et al. 4,448,235 A 5/1984 Silva et al. 4,582,811 A 4/1986 Pucci et al. 4,683,197 A 1/1987 Callard 4,889,818 A 12/1989 Geffard et al. 5,188,365 A 1/1993 Crow et al. 6,410,278 B1 6/2002 Bug et al. 6,761,702 B2 7/2004 Smith 7,288,413 B2 10/2007 Goodlin 7,371,527 B1 5/2008 Beilin et al. 8,560,648 B2 5/2015 Van Engeland et al. 2002/0096469 A1 7/2002 Faulkner FOREIGN PATENT DOCUMENTS 2002/0187476 A1 12/2002 Korolis et al. 2003/0080869 A1 5/2003 Stallings 2003/0096244 A1 5/2003 Ishello et al. 2004/0019289 A1 1/2004 Zhao et al. 2004/0091881 A1 5/2004 Olick et al. 2005/0064401 A1 3/2005 Olick et al. 2005/0075541 A1 4/2005 Chalmers 2005/0244836 A1 11/2005 Tung 2006/0084804 A1 4/2006 Alshbrook et al. 2006/0188919 A1 8/2006 Guo 2006/0210448 A1 9/2006 Wang et al. 2006/0214830 A1 10/2006 Wang et al. 2007/0017015 A1 1/2007 Wang et al. 2007/0072144 A1 7/2007 Wang et al. 2008/0097238 A1 5/2008 Wang et al. 2008/0137716 A1 5/2008 Wang et al. 2008/0221056 A1 10/2008 Wang et al. 2008/0227288 A1 10/2008 Wang et al. 2009/0046158 A1 2/2009 Wang et al. 2010/0184572 A1 9/2010 Wang et al. 2012/0164238 A1 10/2012 Wang et al. 2010/0194723 A1 10/2010 Wang et al. Other References Ohlsson et al., Beta Abnormalities et al. method for colorectal World J Gastroenterol 2006;12(1):1-13 Algarin et al., Cell Screen, Mayo Clin Proc 2006;81(1):1-13 Chen et al., Dose methylation of the a Aug. 3, 2005/0715 Primary Examiner (74) Attorney, Agent, or Firm Mary Ann D. Hec Specifications (57) A method of processing a freshly-collected fecal sample comprising: 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; 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 combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	

-  Steps of Fecal DNA Assay
-  Steps of iFOBT Assay
-  Steps of Both Assays

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.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and
ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer;

the pair of portions obtained by the method of claim 1;

b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Petition, at pp. 1, 25-26, 50-51

Demonstrative Exhibit - Not Evidence

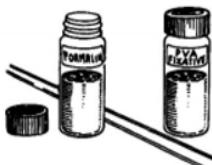
State of the Art: Partitioning Fecal Sample into Different Buffers to Stabilize Different Biomarkers

EX1002 (Whitney Decl.), at p. 42; EX1026 (Melvin), at pp. 7-12

Demonstrative Exhibit - Not Evidence

ADAPTED FROM BROOKE AND GOLDMAN, 1949

NOTE: BOTH SOFT AND FORMED SPECIMENS SHOULD BE SUBMITTED BY THIS METHOD. SPECIMENS MUST BE FRESH WHEN PLACED IN VIALS.



1 THE KIT CONSISTS OF TWO GLASS VIALS (ONE WITH 5% FORMALIN AND ONE WITH PVA-FIXATIVE) AND TWO APPLICATOR STICKS.

2 THE STOOL SHOULD BE PASSED INTO A DRY CONTAINER. URINE SHOULD NOT BE PASSED INTO THE SAME CONTAINER.



3 USING APPLICATOR STICKS, PLACE A QUANTITY OF THE STOOL (ABOUT THE DIAMETER OF A QUARTER) INTO THE VIAL CONTAINING FORMALIN, SCREW CAP ON TIGHTLY.

4 PLACE A SIMILAR QUANTITY INTO THE VIAL CONTAINING THE PVA-FIXATIVE.



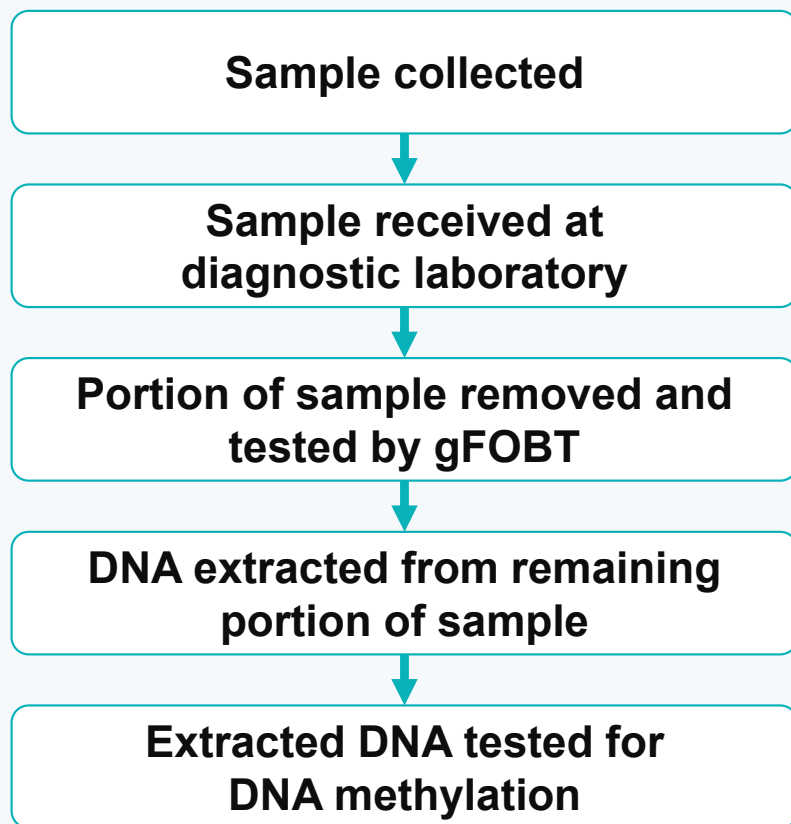
5 THOROUGHLY BREAK UP SPECIMEN IN THE PVA-FIXATIVE, USING APPLICATOR STICKS. SCREW CAP ON TIGHTLY AND SHAKE VIGOROUSLY.

6 PACK THE TWO VIALS SO AS TO PROTECT AGAINST BREAKAGE, ENCLOSE APPROPRIATE IDENTIFICATION, AND MAIL OR DELIVER TO YOUR PUBLIC HEALTH LABORATORY.

Ground I: **Lenhard, Itzkowitz and Vilkin**

Claims 1-9, 11, 14-20

Lenhard's Combined Test



Petition, at pp. 13-14; EX1004 (Lenhard), at pp. 143-45

Demonstrative Exhibit - Not Evidence

Lenhard

Stool samples were collected before cathartic preparation for scheduled surgery or colonoscopy. A period of at least 12 days was respected between previous biopsy examinations of colorectal tumors before stool sampling. Samples were received within 10 hours after defecation at the laboratory, aliquoted, and stored at -80°C .

Fecal Occult Blood Testing

All stool samples were subjected to FOBT (Beckman Coulter, Fullerton, CA) immediately on receipt and before freezing. One drop of the peroxide catalyst was added to the

Isolation of DNA

Stool samples were thawed on ice and DNA was isolated using the QiaAmp DNA Stool Mini-Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, DNA was isolated from stool samples of

Methylation-Specific Polymerase Chain Reaction

MSP is based on a 2-step process.²⁴ First, DNA is treated with sodium bisulfite, which efficiently converts unmethylated cytosine to uracil but leaves methylated cytosines unchanged. Consequently, after treatment, methylated and unmethylated alleles have different sequences. Second, allele-specific primers allow for the specific amplification of methylated and unmethylated alleles.

GASTROENTEROLOGY AND HEPATOLOGY 2005;3:142-149

Stool: A Novel Method for

UTAY,* ROLF SCHAUER,*
* and FRANK T. KOLLIGS*
University of Munich, Munich, Germany; and the *Institute

by disease and precancerous adenomatous lesions
a decrease of CRC-related mortality.¹⁻³ There-
is a strong rationale for screening programs.
scopy, colonoscopy, and fecal occult blood test
alone or in combination with endoscopy, are
needed for screening of the average-risk popula-
However, FOBT, when applied regularly, can
CRC mortality by only 15%-33%.⁴⁻⁷ Compli-
with endoscopic screening is not satisfactory. Over-
1998, only 37% of eligible adults in the United
had been screened for CRC in the previous 3

ection of tumor-derived genetic changes in stool
missing new approach for CRC screening. Stud-
lished so far focus on the detection of muta-
in oncogenes,⁸⁻¹⁶ and tumor-suppressor
as well as changes in microsatellite mark-
Two recent studies required the analysis of up to
recent genetic markers¹⁷ or 144 aliquots from
stool¹⁸ for the detection of 91% and 61% of
and 82% and 50% of adenomas ≥ 1 cm, re-
sively. This precludes their application for routine
throughout screening owing to high labor inten-
costs. The identification of new markers with
sensitivities and specificities is needed. So far,
attention has been paid to DNA hypermethy-
as a potential stool marker for CRC.

ylation of CpG islands of promoters leads to
ing of transcription of the affected gene.^{22,23}
lation-specific polymerase chain reaction (PCR)
has been used successfully to detect DNA
in primary tumors and in various body
The feasibility of amplification of methylated

ations used in this paper: CI, confidence interval; CRC, colo-
rectal cancer; FOBT, fecal occult blood test; HCL, hypermethylated in-
L, MSP, methylation-specific PCR; PCR, polymerase chain re-

2005 by the American Gastroenterological Association
1542-3565/05/\$30.00
Pii: 10.1053/j.gie.2004.06.024X
Genoscopy Exhibit 1004, Page 1

Lenhard Discloses a Test that Combines a Fecal DNA Assay and a FOBT

tissue.³¹ We show that HIC1 promoter methylation can be detected frequently and with high specificity in stool samples from patients with CRCs. The combination of HIC1 methylation analysis with FOBT allowed for the detection of two thirds of CRCs.

more adenomas (31% vs. 8%, $P = .14$). In addition, specificity of the HIC1 assay was higher (97.9% vs. 89.9%, $P = .102$). The combination of both assays resulted in increased detection rates for CRCs (65%; 95% CI, 44%–83%). Although the combined test detected all localized cancers, no increase in the sensitivity for adenoma was seen.

The use of the DNA methylation marker, HIC1 promoter methylation, allowed for the highly specific detection of CRCs in 42% and of adenomas ≥ 1 cm in 31% of cases. Interestingly, sensitivity was quite high for early cancers and higher for adenoma than FOBT. The combination of HIC1 MSP with FOBT resulted in the detection of almost two thirds of CRCs.

Table 4. Positivity Rates of the Fecal DNA Assay, FOBT, and Combination of the Tests

	HIC1		FOBT		<u>Combined</u>	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
All cancers	11/26 (42)	23%–63%	9/26 (35)	17%–56%	<u>17/26 (65)</u>	44%–83%
Localized cancers	4/6 (67)	22%–96%	2/6 (33)	4%–77%	6/6 (100)	12%–188% ^a
Advanced cancers	5/20 (25)	9%–49%	7/20 (35)	15%–59%	11/20 (55)	32%–77%
Rectum and sigmoid colon	5/13 (39)	14%–68%	5/13 (39)	14%–68%	9/13 (69)	39%–91%
From descending colon to cecum	6/13 (46)	19%–75%	4/13 (31)	14%–68%	8/13 (62)	39%–91%
Asymptomatic cases	4/10 (40)	12%–74%	3/10 (30)	7%–65%	6/10 (60)	26%–88%
Symptomatic cases	7/16 (44)	20%–70%	6/16 (38)	15%–65%	11/16 (69)	41%–89%
Adenomas	4/13 (31)	9%–61%	1/13 (8)	.2%–36%	4/13 (31)	9%–61%

^aEstimated by normal approximation of Poisson distribution.

Petition, at pp. 13-14, 21, 25-26; EX1004 (Lenhard), at pp. 143, 146-47

Lenhard's Combined Test Reduces False Negatives for CRC Detection

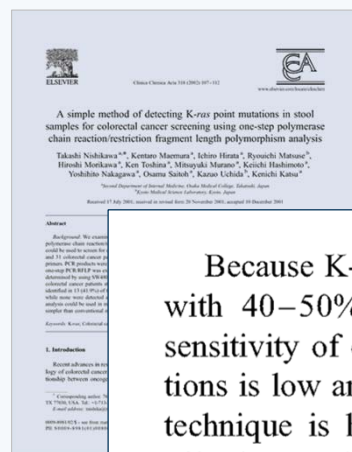
CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2005;3:142-147							
Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer							
KONSTANZE LENHARD,* GUIDO T. BOMMER,* SILKE ASUTAY,* ROLF SCHAUER,* THOMAS BRABLETZ,* BURKHARD GÖKE,* ROLF LAMERZ,* and FRANK T. KOLLIGS*							
*Department of Medicine II, and *Department of Surgery, Klinikum Grosshadern, University of Munich, Munich, Germany; and the *Institute of Pathology, University of Erlangen, Erlangen, Germany							
Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of tumors, usually multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high throughput screening. Therefore, markers with high sensitivity and good specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. Methods: Methylation-specific polymerase chain reaction (PCR) was performed to analyze hypermethylated in cancer 1 (HIC1) promoter methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma ≥ 1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colon. Results: Ninety-seven percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 31% of the samples from patients with colorectal adenoma ≥ 1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the fecal DNA from patients with endoscopically normal colon or hyperplastic polyps. Conclusions: The epigenetic marker HIC1 promoter methylation carries high potential for the remote detection of CRCs. We postulate that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRCs and adenomas in fecal samples from affected patients. Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.¹ About 40% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.² The majority of CRCs follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.³ Detection of early disease and precancerous adenomatous lesions leads to a decrease of CRC-related mortality.⁴⁻⁷ Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood (FOBT) alone or in combination with endoscopy, recommended for screening of the average-risk population.⁸ However, FOBT, when applied regularly, decrease CRC mortality by only 15%-35%.⁹⁻¹² Compliance with endoscopic screening is not satisfactory. Of all, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous years.⁹ Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,¹⁰⁻¹⁶ and tumor-suppressor genes,¹⁷⁻¹⁹ as well as changes in microsatellite markers.²⁰ Two recent studies required the analysis of up to 15 different genetic markers²¹ or 144 aliquots from each stool¹⁸ for the detection of 91% and 61% cancers and 82% and 50% of adenomas ≥ 1 cm, respectively. This precludes their application for routine high throughput screening owing to high labor intensity and costs. The identification of new markers with high sensitivities and specificities is needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC. Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.²² Methylation-specific polymerase chain reaction (PCR) (MSP)²³ has been used successfully to detect DNA methylation in primary tumors and in various body fluids.²⁴ The feasibility of amplification of methylated DNA from stool samples has been demonstrated.²⁵							
Table 1. Clinicopathologic Data and HIC1 Methylation Status of Patients With CRC							
No.	Sex	Age (y)	UICC stage	Localization	Symptoms ^a	HIC1 methylation	FOBT
1	M	71	IV	Sigmoid colon/rectum	1	—	—
2	F	75	III	Sigmoid colon	1,3	—	—
3	M	66	IV	Descending/sigmoid colon	0	—	—
4	M	69	II	Ascending colon	0	+	—
5	M	65	III	Transverse colon	0	+	+
6	M	56	IV	Rectum	1	+	—
7	M	63	IV	Ascending colon	2	—	—
8	F	72	III	Cecum	2	+	+
9	M	74	III	Sigmoid colon/rectum	0	—	+
10	M	68	IV	Rectum	3	+	+
11	M	66	IV	Rectum	1	—	+
12	M	75	III	Cecum	0	—	—
13	M	71	IV	Sigmoid colon	0	+	—
14	M	67	II	Hepatic flexure	3	+	—
15	F	72	IV	Sigmoid colon	1,2	+	—
16	M	59	II	Rectum	0	+	—
17	M	59	II	Rectum	0	—	+
18	M	53	III	Descending colon	0	—	—
19	F	55	IV	Rectum	2	—	+
20	F	61	IV	Sigmoid colon	2,3	—	—
21	M	61	IV	Rectum	0	—	—
22	M	80	II	Transverse colon	2	+	—
23	F	53	IV	Ascending colon	1,3	—	—
24	M	63	I	Cecum	4	NA	+
25	M	76	III	Transverse colon	2	—	+
26	F	60	IV	Ascending colon	1	+	—

Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; FOBT, fecal occult blood test; HIC1, hypermethylated in cancer 1; MSP, methylation-specific PCR; PCR, polymerase chain reaction.							
© 2005 by the American Gastroenterological Association 1542-3566/05/\$30.00 DOI: 10.1053/j.gastro.2004.06.024X Gastroenterology Exhibit 1004, Page 1							
UICC, International Union Against Cancer; M, male; F, female; NA, no amplification.							
^a0, no symptoms; 1, change in bowel habits; 2, blood admixed with stool; 3, abdominal pain; 4, anemia.							

Petition, at pp. 13-14, 21, 25-26; EX1004 (Lenhard), at p. 143

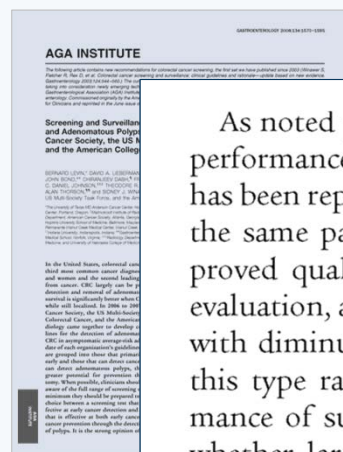
Demonstrative Exhibit - Not Evidence

Combining Fecal DNA Tests with FOBT was a Known Strategy to Improve Fecal CRC Tests



Nishikawa

Because *K-ras* point mutations are only associated with 40–50% of colorectal cancers, the detection sensitivity of one-step PCR/RFLP analysis for mutations is low among cancers as a whole. However, the technique is highly specific, noninvasive, and cost-effective, and should provide a more sensitive and specific tool for mass screening of colorectal cancer than is currently available, especially if used in combination with fecal occult blood testing and other methods for detecting genetic abnormalities.



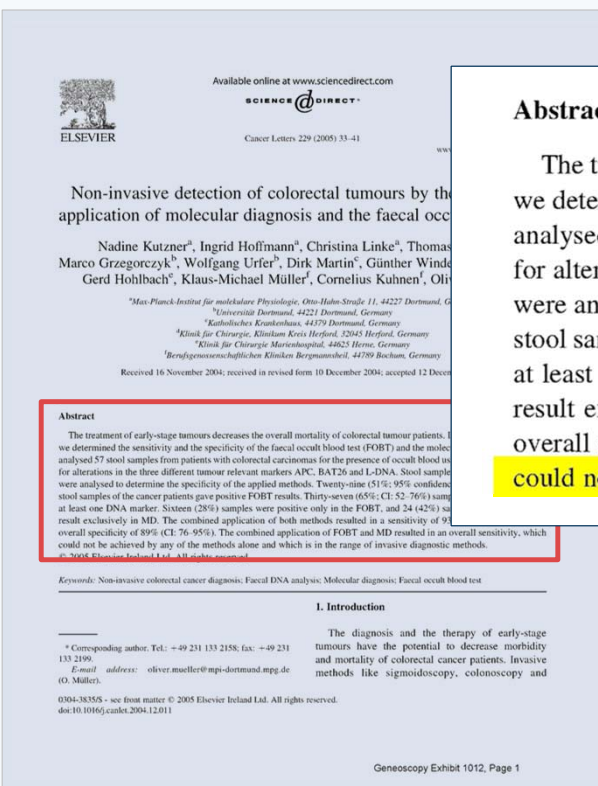
Levin

As noted previously, the most informative data on the performance of sDNA testing is from version 1.0, which has been replaced with version 1.1; the newer version uses the same panel of markers but is reported to have improved quality.^{63,70} Newer versions are currently under evaluation, and are reported to have improved sensitivity, with diminution of specificity. The evolution of tests of this type raises important questions as to how performance of successive iterations should be evaluated and whether large prospective studies of asymptomatic patients with follow-up CSFY among all participants are required. Another question worthy of consideration is whether or not including a sensitive gFOBT or FIT at the time of testing would improve sensitivity without adversely affecting specificity. In a recent retrospective analysis of stool samples from patients with CRC and donor controls, combined results from a standard gFOBT and a panel of DNA markers (APC, BAT26, and L-DNA) resulted in a combined sensitivity for cancer of 93% and specificity of 89%.⁷⁶

Petition, at pp. 8-9, 48-50;
EX1011 (Nishikawa), at p. 112; EX1013 (Levin), at p. 1578

Combining Fecal DNA Tests with FOBT Was a Known Strategy to Improve Fecal CRC Tests

Kutzner



Abstract

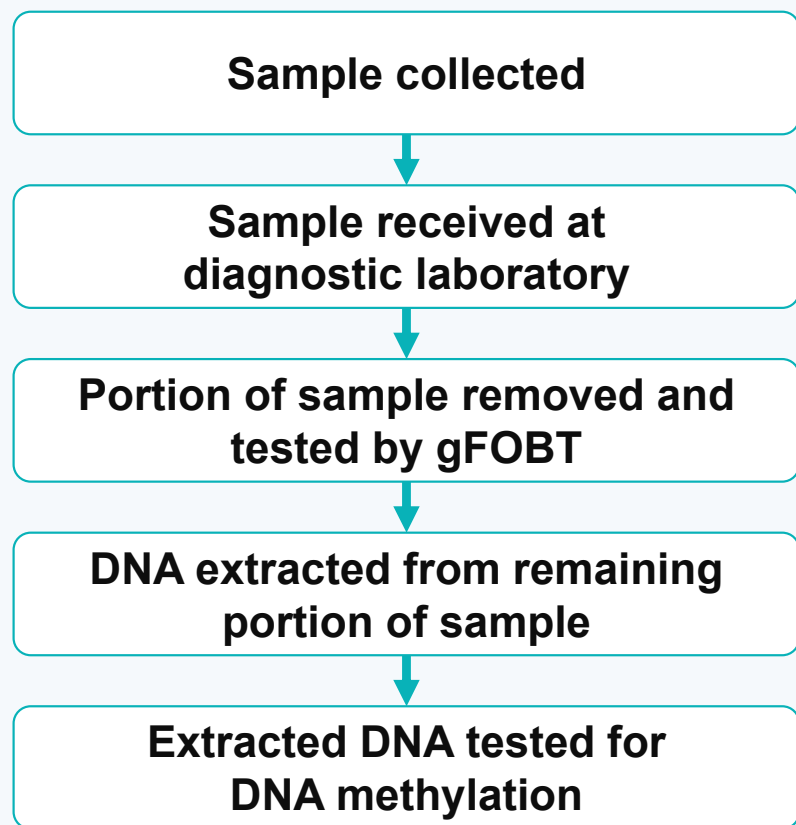
The treatment of early-stage tumours decreases the overall mortality of colorectal tumour patients. In this retrospective study we determined the sensitivity and the specificity of the faecal occult blood test (FOBT) and the molecular diagnosis (MD). We analysed 57 stool samples from patients with colorectal 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.

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.

Petition, at pp. 8-9, 48-50;
EX1012 (Kutzner), at Abstract, p. 40

Demonstrative Exhibit - Not Evidence

A POSA Would Be Motivated to Apply Known Techniques to Improve Lenhard's Combined Test



POSA would be motivated to incorporate Itzkowitz's sample collection process to facilitate at-home sample collection

POSA would be motivated to replace gFOBT with Vilkin's superior iFOBT to increase patient convenience and improve assay performance

Petition, at pp. 20-26; EX1004 (Lenhard); EX1005 (Vilkin); EX1006 (Itzkowitz)

Incorporation of Itzkowitz's Sample Collection and Vilkin's iFOBT into Lenhard's Combined Test Results in the Claimed Invention

 US 11,634,781 B2	
(12) United States Patent Louwagie	(10) Patent No.: US 11,634,781 B2 (45) Date of Patent: *Apr. 25, 2023
(54) FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD (71) Applicant: Exact Sciences Corporation, Madison, WI (US) (72) Inventor: Joost Louwagie , Domach (CH) (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(d) by 0 days. This patent is subject to a terminal disclaimer. (21) Appl. No.: 17/936,335 (22) Filed: Sep. 28, 2022 (65) Prior Publication Data US 2023/0048152 A1 Feb. 16, 2023 Related U.S. Application Data (63) Continuation of application No. 15/034,607, filed on Jun. 27, 2017, which is a continuation of application No. 15/010,436, filed on Jan. 29, 2016, now abandoned, which is a continuation of application No. 13/147,570, filed as application No. PCT/GB2010/000180 on Feb. 3, 2010, now abandoned. (69) Provisional application No. 61/149,581, filed on Feb. 3, 2009. (51) Int. Cl. C12Q 1/6886 (2018.01) G01N 33/574 (2006.01) U.S. Cl. CPC — C12Q 1/6886 (2013.01); GBIN 33/57446 (2013.01); C12Q 2600/156 (2013.01); C12Q 2600/154 (2013.01); GBIN 2333/805 (2013.01); GBIN 2600/50 (2013.01); GBIN 2600/55 (2013.01) (58) Field of Classification Search CPC — C12Q 1/68 ; C12Q 2600/154 ; C12Q 2600/156 ; GBIN 33/57446 ; GBIN 2333/805 See application file for complete search history. (56) References Cited U.S. PATENT DOCUMENTS 4,447,975 A 3/1984 Gillette et al. 4,444,231 A 3/1984 Silva et al. 4,582,811 A 4/1986 Pucci et al. 4,683,197 A 1/1987 Gallat 4,889,818 A 12/1989 Geffard et al. 5,188,365 A 1/1993 Crow et al. 6,410,278 B1 6/2002 Bug et al. 6,761,702 B2 7/2004 Smith 7,288,413 B2 10/2007 Goodwin 7,371,527 B1 5/2008 Beutin et al. 8,560,646 B2 5/2015 Van Engeland et al. 2002/099649 A1 7/2002 Faulkner 2002/0187476 A1 12/2002 Korolis et al. 2003/0080869 A1 5/2003 Stallings 2003/0096244 A1 5/2003 Eshel et al. 2004/0019289 A1 1/2004 Zhao et al. 2004/0091881 A1 5/2004 Ock et al. 2005/0064401 A1 3/2005 Ock et al. 2005/0075481 A1 4/2005 Chalmers 2005/0248836 A1 11/2005 Tsang 2006/0084054 A1 4/2006 Alshbrook et al. 2006/0189919 A1 8/2006 Gao 2006/0210448 A1 9/2006 Hsu et al. 2006/0214830 A1 10/2006 Hsu et al. 2007/0017015 A1 1/2007 Hsu et al. 2007/0072714 A1 7/2007 Hsu et al. 2008/0097238 A1 6/2008 Hsu et al. 2008/0137716 A1 5/2008 Hsu et al. 2008/0221056 A1 10/2008 Hsu et al. 2008/0277208 A1 11/2008 Hsu et al. 2010/0184572 A1 8/2010 Hsu et al. 2012/0164238 A1 8/2012 Hsu et al. 2010/0194723 A1 1/2010 Hsu et al. FORE EP 06 EP 03 EP 06 FR 25 WO WO 1996 WO WO 1997 WO WO 2000 WO WO 2004 WO WO 2004 WO WO 2004 G Ohlsson et al., Beta Absorption of fat method for colorectal World J Gastroenterol 14(1):1-5 (2006.01) Alghamdi et al., Cell Screen, Mayo Clin Proc 78(1):1-5 (2003.01) Muller et al., J Clin Invest 113(1):1-5 (2003.01) Chen et al., Dose methylation of the a Aug. 3, 2005/0715 Primary Examiner (74) Attorney, Agent, or Solicitor Mary Ann D. Hsu Specification	

-  Steps of Fecal DNA Assay
-  Steps of iFOBT Assay
-  Steps of Both Assays

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.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and
ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

the pair of portions obtained by the method of claim 1;

b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Petition, at pp. 1, 26-27

Demonstrative Exhibit - Not Evidence

Itzkowitz's Fecal DNA Test

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;

d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:

ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Itzkowitz

Sample Collection

To avoid any possible effect of the colonoscopic bowel preparation on test results, each subject provided a single stool sample approximately 6–14 days after colonoscopy. In the case of patients with CRC, the sample was provided before beginning the presurgical bowel preparation. Subjects were given detailed instructions and a special stool collection kit that is mounted on the toilet bowl. Immediately after defecation, subjects added 250 mL of a DNA-stabilizing buffer⁷ to a stool specimen of at least 50 g. Only 10 patients provided less than 50 g of stool, and, of these, 3 subsequently provided an adequate second specimen. The specimen was shipped at room temperature overnight using a coded identifier provided by an external clinical research organization (Carestat Inc., Newton, MA) to keep the laboratory blinded to the clinical source. The

These improvements of better DNA stabilization, enhanced DNA extraction, and use of gene-specific methylation have been incorporated into a second-generation fecal DNA test (version 2). The purpose of the present study was to determine the sensitivity and specificity of the newer version 2 assay for detection of CRC.

Petition at pp. 16-17, 27-28, 33-35; EX1006 (Itzkowitz), at p. 112

Advantages of Using Itzkowitz's At-Home Sample Collection Process for Fecal DNA Tests Were Known

[illegible]

Since that time, pilot studies have shown that several technical and conceptual advances could improve fecal DNA testing. First, adding a DNA-stabilizing buffer to the stool immediately on defecation was shown to prevent DNA degradation for several days and enhance the performance of DIA.⁷ Second,

* * *

the DNA degradation. This increase in sensitivity for the full version 1 panel was achieved at the expense of a relatively minor decrease in specificity from 94.4% to 89.3%. Thus, even with the original markers, improved DNA preservation and extraction from stool resulted in a better screening test.

Itzkowitz

[illegible]

mutant DNA available for the amplification reaction. Despite the apparent stability of the point mutation markers to DNA degradation, it is nevertheless desirable to mitigate loss of DNA if possible. It has recently been shown¹⁰ that to maximize clinical sensitivity of fecal DNA assays, it is important to maximize the recovery of otherwise scarce amounts of human DNA.

Olson

Vilkin's Automated iFOBT

Vilkin

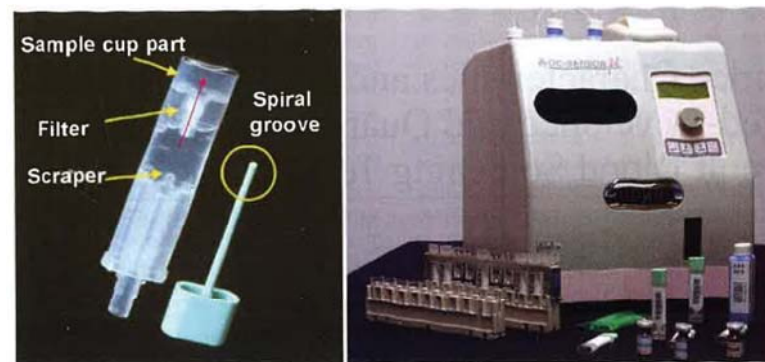
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:

- 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

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

- a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and

- b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;



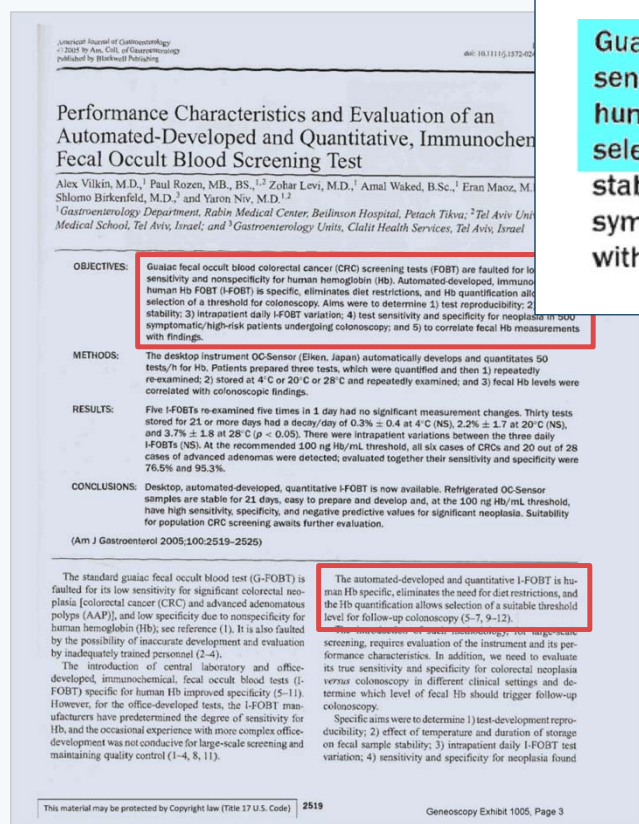
Fecal Sampling

The fecal test sampling device is shaped like a small test tube with the fecal probe inserted into it and sealing it. The probe has a serrated tip, which is poked into the stool and then pushed back into the tube, past a scraper, and through a membrane into the sample cup. These remove most of the excess feces and leave the stool collected (about 10 mg) into the serrations (Fig. 1). The tip is then put in a closed amount of Hb stabilizing buffer. Each sample tube has a unique bar code and before preparing the sample the patient writes his name and date on the tube. The examinee is requested to prepare three consecutive daily samples during the week before colonoscopy examination. Samples are double-closed in zip-lock bags and kept in the refrigerator until returned to the developing laboratory where they are also kept at 4°C until development.

Petition, at pp. 14-16, 27-31, 34; EX1005 (Vilkin), at p. 2520

Vilkin Teaches Advantages of iFOBT over gFOBT

Vilkin



Guaiac fecal occult blood colorectal cancer (CRC) screening tests (FOBT) are faulted for low sensitivity and nonspecificity for human hemoglobin (Hb). Automated-developed, immunochemical, human Hb FOBT (i-FOBT) is specific, eliminates diet restrictions, and Hb quantification allows selection of a threshold for colonoscopy. Aims were to determine 1) test reproducibility; 2) test stability; 3) intrapatient daily i-FOBT variation; 4) test sensitivity and specificity for neoplasia in 500 symptomatic/high-risk patients undergoing colonoscopy; and 5) to correlate fecal Hb measurements with findings.

The automated-developed and quantitative i-FOBT is human Hb specific, eliminates the need for diet restrictions, and the Hb quantification allows selection of a suitable threshold level for follow-up colonoscopy (5-7, 9-12).

The introduction of the instrument-developed, quantitative, fecal occult blood testing allows the physician to choose the optimal fecal Hb threshold level that triggers a follow-up colonoscopy. To validate this premise, we performed

Petition, at pp. 21-23; EX1005 (Vilkin), at Abstract, pp. 2519, 2524

Demonstrative Exhibit - Not Evidence

Advantages of iFOBT over gFOBT Were Known in the Art

Young

Population participation is essential for cancer detection [3]. FIT technology simplifies the testing process, removes the need for diet and drug restrictions, provides for preferred and more acceptable stool-sampling methods such as brushes or probes rather than a wooden spatula, and is achieved while collecting fewer fecal samples. Most branded versions of FIT require fewer than three fecal samples, the recommended number for GFOBT. Removal of dietary restrictions has been shown to enhance participation in screening with FIT relative to GFOBT, in one study by 28% [10]. Changing to a brush-sampling method also simplifies the process and enhances participation by 30%. Together, these two advances increase population participation by 66% [10].

* * *

Obviously, FITs overcome most of the disadvantages presented by GFOBT, are superior to GFOBT in terms of participation as well as performance and should replace GFOBT in two-step screening [14, 16].

Guittet

Evidence in favour of the use of the I-FOBT over the G-FOBT is increasing. I-FOBT tests have no dietary or medication restrictions. These tests have superior sensitivity and specificity, the gain being more important for high risk adenomas than for cancers. They also have a higher compliance rate^{23 26 27} and the automated reading technology allows the choice of the ideal positivity rate. As suggested in recent reviews,^{5 7} it is time to give colorectal cancer screening a new future by using I-FOBT instead of G-FOBT.

Petition, at pp. 22-23; EX1024 (Young), at pp. 29-30; EX1010 (Guittet), at p. 214

Demonstrative Exhibit - Not Evidence

Incorporation of Itzkowitz's Sample Collection and Vilkin's iFOBT into Lenhard's Combined Test Results in the Claimed Invention

	
US 11,634,781 B2	
(12) United States Patent Louwagie	(10) Patent No.: US 11,634,781 B2 (45) Date of Patent: *Apr. 25, 2023
(54) FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD (71) Applicant: Exact Sciences Corporation, Madison, WI (US) (72) Inventor: Jost Louwagie, Domach (CH) (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days. This patent is subject to a terminal disclaimer. (21) Appl. No.: 17/936,335 (22) Filed: Sep. 28, 2022 (65) Prior Publication Data US 2023/0048152 A1 Feb. 16, 2023 Related U.S. Application Data (63) Continuation of application No. 15/034,607, filed on Jun. 27, 2017, which is a continuation of application No. 15/010,436, filed on Jan. 29, 2016, now abandoned, which is a continuation of application No. 13/147,570, filed as application No. PCT/US2010/000180 on Feb. 3, 2010, now abandoned. (69) Provisional application No. 61/149,581, filed on Feb. 3, 2009. (51) Int. Cl. C12Q 1/6886 (2018.01) GOIN 1/8574 (2006.01) (52) U.S. Cl. CPC — C12Q 1/6886 (2013.01); GOIN 1/8574 (2006.01); C12Q 2600/156 (2013.01); C12Q 2600/152 (2013.01); C12Q 2600/158 (2013.01); GOIN 2333/805 (2013.01); GOIN 2600/50 (2013.01); GOIN 2600/52 (2013.01) (58) Field of Classification Search CPC — C12Q 1/68; C12Q 2600/154; C12Q 2600/156; GOIN 1/8574; GOIN 2333/805 See application file for complete search history. (56) References Cited U.S. PATENT DOCUMENTS 4,447,975 A 3/1984 Gillette et al. 4,443,231 A 3/1984 Silva et al. 4,582,811 A 4/1986 Pucci et al. 4,683,197 A 1/1987 Gallat 4,889,818 A 12/1989 Geffard et al. 5,188,365 A 1/1993 Crow et al. 6,410,278 B1 6/2002 Burg et al. 6,761,702 B2 7/2004 Smith 7,288,413 B2 10/2007 Goodwin 7,371,527 B1 5/2008 Baylin et al. 8,560,646 B2 5/2015 Van Engeland et al. 2002/099649 A1 7/2002 Faulkner FOREIGN PATENT DOCUMENTS 2002/0187476 A1 12/2002 Korolis et al. 2003/0080869 A1 5/2003 Stallings 2003/0096244 A1 5/2003 Eshel et al. 2004/0019289 A1 1/2004 Zhao et al. 2004/0091881 A1 5/2004 Ock et al. 2005/0064401 A1 3/2005 Ock et al. 2005/0075481 A1 4/2005 Chalmers 2005/0248836 A1 11/2005 Tung 2006/0084054 A1 4/2006 Alshbrook et al. 2006/0189019 A1 8/2006 Gao 2006/0210448 A1 9/2006 Wang et al. 2006/0214830 A1 10/2006 Wang et al. 2007/0017015 A1 1/2007 Wang et al. 2007/0072714 A1 7/2007 Wang et al. 2008/0097738 A1 6/2008 Wang et al. 2008/0137714 A1 5/2008 Wang et al. 2008/0221056 A1 10/2008 Wang et al. 2008/0277288 A1 11/2008 Wang et al. 2009/0046058 A1 2/2009 Wang et al. 2010/0184572 A1 9/2010 Wang et al. 2012/0164238 A1 8/2012 Wang et al. 2010/0194723 A1 9/2010 Wang et al. Other References Ohlsson et al., Beta Abnormalities et al. method for colorectal World J Gastroenterol 14(1):1-5 (2008). Algarin et al., Cell Screen, Mayo Clin Proc 78(1):1-5 (2003). Algarin et al., Cell Screen, Mayo Clin Proc 78(1):1-5 (2003). Chen et al., Dose methylation of the a Aug. 3, 2005/071515 Primary Examiner (74) Attorney, Agent, or Patent Attorney Mary Ann D. Hie (57) A method of process comprising: removing and adding the reagent to the sample, and the removed portion comprises: stabilizing buffer. Specifications	

-  Steps of Fecal DNA Assay
-  Steps of iFOBT Assay
-  Steps of Both Assays

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.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and
ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

the pair of portions obtained by the method of claim 1;

b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Petition, at pp. 1, 26-27

Demonstrative Exhibit - Not Evidence

Exact's Attack on Motivation to Combine Has No Merit



“Our precedent... does not require that the motivation be the best option, only that it be a suitable option from which the prior art does not teach away.”

Gen. Elec. Co. v. Raytheon Techs. Corp., 983 F.3d 1334 (Fed. Cir. 2020),
quoting *Par Pharm., Inc. v. Twi Pharms., Inc.*, 773 F.3d 1186, 1197-98 (Fed. Cir. 2014)

more adenomas (31% vs. 8%, $P = .14$). In addition, specificity of the HIC1 assay was higher (97.9% vs. 89.9%, $P = .102$). The combination of both assays resulted in increased detection rates for CRCs (65%; 95% CI, 44%–83%). Although the combined test detected all localized cancers, no increase in the sensitivity for adenoma was seen.

ing. Combination of HIC1 with a few additional sensitive and specific methylation markers may allow for the highly sensitive and specific stool-based detection of CRCs and adenomas. Evaluation of other genes known to

Reply, at pp. 6-7; EX1004 (Lenhard), at pp. 143, 146-148

Demonstrative Exhibit - Not Evidence

Exact's Attack on Motivation to Combine Has No Merit

Sensitivity, not specificity, is of primary importance for fecal CRC assays.

- In touting the improved sensitivity of its combined assay, Lenhard does not characterize its 89% specificity as a negative feature
- In Itzkowitz, Exact characterizes version of their fecal DNA test that showed a similar improvement to sensitivity and loss of specificity to Lenhard's combined assay as a "better screening test".
- The specificity of Exact's preferred screening test in Itzkowitz was only 82%
- A POSA would be motivated to improve the specificity of Lenhard's assay by replacing its gFOBT with Vilkin's iFOBT

Itzkowitz

viduals.⁶ By using improved methods for stabilizing DNA during specimen transport, and better extraction of DNA from stool, we now find that the sensitivity for the version 1 marker panel is increased from 52% to 72.5%. This improvement was a direct result of an increase in DIA sensitivity from 3% to 65%, confirming the observations of Olson et al⁷ that the low sensitivity of DIA in the previous multicenter study was as result of the DNA degradation. This increase in sensitivity for the full version 1 panel was achieved at the expense of a relatively minor decrease in specificity from 94.4% to 89.3%. Thus, even with the original markers, improved DNA preservation and extraction from stool resulted in a better screening test.

Reply, at pp.9-10; EX1006 (Itzkowitz), at pp. 111, 116;
EX1005 (Vilkin), at p. 2523; EX2008 (Imperiale), at p. 1295

Exact's Attack on Motivation to Combine Has No Merit

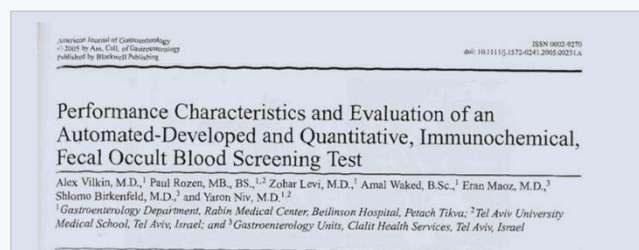
Levin

- Patent Owner argues Levin contradicts that there were known advantages of iFOBT over gFOBT based on statement that there “were no clear patterns of superior performance [...] between a high-sensitivity guaiac-based test (Hemoccult SENSE) and a variety of FIT.” (POR, p.36)
- Patent Owner’s argument ignores the multiple advantages of iFOBT over gFOBT which Levin acknowledges.
- Patent Owner’s argument ignores that Lenhard did not use the high-sensitivity gFOBT referenced in Levin.

FIT has several technological advantages when compared with gFOBT. FIT detects human globin, a protein that along with heme constitutes human hemoglobin. Thus, FIT is more specific for human blood than guaiac-based tests, which rely on detection of peroxidase in human blood and also react to the peroxidase that is present in dietary constituents such as rare red meat, cruciferous vegetables, and some fruits.⁵³ Further, unlike gFOBT, FIT is not subject to false-negative results in the presence of high-dose vitamin C supplements, which block the peroxidase reaction. In addition, because globin is degraded by digestive enzymes in the upper gastrointestinal tract, FIT also are more specific for lower gastrointestinal bleeding, thus improving their specificity for CRC. Finally, the sample collection for some variants of FIT are less demanding of patients than gFOBT, requiring fewer samples or less direct handling of stool.

Reply, at pp. 13-14; Levin (EX1013), at p. 1576

Exact's Attack on Motivation to Combine Has No Merit



- Vilkin does not require fecal collection on three consecutive days

Vilkin

Specific aims were to determine 1) test-development reproducibility; 2) effect of temperature and duration of storage on fecal sample stability; 3) inpatient daily I-FOBT test variation; 4) sensitivity and specificity for neoplasia found in persons also undergoing colonoscopy; and 5) to correlate fecal Hb levels with clinical findings.

plasia [colorectal cancer (CRC) and advanced adenomatous polyps (AAP)], and low specificity due to nonspecificity for human hemoglobin (Hb); see reference (1). It is also limited by the possibility of inaccurate development and evaluation by inadequately trained personnel (2-4).

The introduction of central laboratory and office-developed, immunochemical, fecal occult blood tests (I-FOBT) specific for human Hb improved specificity (5-11). However, for the office-developed tests, the I-FOBT manufacturers have predetermined the degree of sensitivity for Hb, and the occasional experience with more complex office-developed was not conducive for large-scale screening and maintaining quality control (1-4, 8, 11).

the Hb quantification allows selection of a suitable threshold level for follow-up colonoscopy (5-7, 9-12).

The introduction of such methodology, for large-scale screening, requires evaluation of the instrument and its performance characteristics. In addition, we need to evaluate its true sensitivity and specificity for colorectal neoplasia versus colonoscopy in different clinical settings and determine which level of fecal Hb should trigger follow-up colonoscopy.

Specific aims were to determine 1) test-development reproducibility; 2) effect of temperature and duration of storage on fecal sample stability; 3) inpatient daily I-FOBT test variation; 4) sensitivity and specificity for neoplasia found

At present, we will continue with collecting three annual fecal tests, as we have confirmed the additive clinical value of repeated occult blood testing in this high-risk population. This is in contrast to the annual 2-day, average-risk population I-FOBT collection, as routinely used in Japan, United States, and Australia and 1-day biennial testing as performed in Italy (11, 12, 16-18).

The decision on the number of fecal samples to be obtained and optimal fecal Hb threshold chosen for screening an average risk population will also involve an evaluation of cost-benefit. This will require a prospective colonoscopy

Reply, at pp. 10-12; EX1005 (Vilkin), at pp. 2519-20, 2524

Demonstrative Exhibit - Not Evidence

Exact's Attack on Motivation to Combine Has No Merit

Exact distorts Vilkin's sensitivity

anything else. Vilkin discloses **100%** sensitivity for CRC. EX1005 at 7 (Table 3).

It is impossible to improve on 100% sensitivity. Accordingly, if, as Petitioner contends, the potential for increased sensitivity for CRC was the most important consideration, there would have been no reason to combine Vilkin. A POSA

Sur-Reply

Vilkin

Table 2. Sensitivity and Specificity for Significant Colorectal Neoplasia, (N = 34)* at Differing Fecal Hb Levels (N = 500)†

Fecal Hb (ng/mL)	Sensitivity (%)	Specificity (%)
50	79.4	89.7
75	76.5	93.3
100	76.5	95.3
125	70.6	95.7
150		
200		

*Six colorectal cancers and
†Utilizing the highest of the

***Six colorectal cancers**

Table 3. Sensitivity for Significant Colorectal Neoplasia (N = 34)* for Each I-FOBT† Prepared

I-FOBT Positive	Cancer (%)	Cancer + AAP (%)
Sample 1	83.3	52.9
Sample 2	66.7	52.9
Sample 3	100	61.8
Samples 1 and 2	83.3	64.7
Samples 1, 2, and 3	100	76.5

Six colorectal cancers (CRC) and 2
At the 100 ng Hb/mL threshold.

***Six colorectal cancers**

Sur-Reply, at p. 2;
EX1005 (Vilkin),
at p. 2523

Exact's Attack on Motivation to Combine Has No Merit

Equal sensitivity for adenomas would not discourage Lenhard's combined test

Levin

- As of the priority date, cancer detection was of primary importance in fecal CRC assays.
- The 2008 American Cancer Society guidelines confirms that adenoma screening is not the primary goal of fecal DNA tests, which “primarily are effective at identifying CRC.”
- In Itzkowitz, Exact assesses performance of versions its fecal DNA assay based solely on their ability to detect cancer, never testing for adenoma detection.

Individuals and health care professionals should also understand that screening tests for CRC broadly fall into 2 categories. In one category are the fecal tests (ie, gFOBT, FIT, and sDNA), which are tests that primarily are effective at identifying CRC. Some premalignant adenomatous polyps may be detected, providing an opportunity for polypectomy and the prevention of CRC, but the opportunity for prevention is both limited and incidental and is not the primary goal of CRC screening with these tests. In the second category are the partial or full structural exams (ie, FSIG, CSPY, DCBE, and CTC)⁴⁴ which are tests that are effective at detecting cancer and premalignant adenomatous polyps. These tests differ in complexity and accuracy for the detection of CRC and advanced neoplasia. When performed properly, each of these structural exams has met the standard of detecting at least half of prevalent or incident cancers at the time of testing.

Reply, at p. 8; EX1080 (Whitney 2nd Decl.) ¶¶ 19-22, 52-54; EX1006 (Itzkowitz); EX1013 (Levin), at pp. 1572-73

Exact's Attack on Motivation to Combine Has No Merit

A POSA would not have been dissuaded from using Lenhard's combined test by cost and complexity.

- Using Itzkowitz's sample collection process and replacing Lenhard's gFOBT with Vilkin's iFOBT increases patient convenience by facilitating at-home sample collection.
- The iFOBT sample collection process was known to be more convenient for patients than the corresponding gFOBT process.
- iFOBT tests were well established in the art and relatively inexpensive.

Reply, at pp.14-15; EX1024 (Young), at p. 29; EX1013 (Levin), at p.1576

Population participation is essential for cancer detection [3]. FIT technology simplifies the testing process, removes the need for diet and drug restrictions, provides for preferred and more acceptable stool-sampling methods such as brushes or probes rather than a wooden spatula, and is achieved while collecting fewer fecal samples. Most branded versions of FIT require fewer than three fecal samples, the recommended number for GFOBT. Removal of dietary restrictions has been shown to enhance participation in screening with FIT relative to GFOBT, in one study by 28% [10]. Changing to a brush-sampling method also simplifies the process and enhances participation by 30%. Together, these two advances increase population participation by 66% [10].

Young

block the peroxidase reaction. In addition, because globin is degraded by digestive enzymes in the upper gastrointestinal tract, FIT also are more specific for lower gastrointestinal bleeding, thus improving their specificity for CRC. Finally, the sample collection for some variants of FIT are less demanding of patients than gFOBT, requiring fewer samples or less direct handling of stool.

Levin

Exact's Attack on Motivation to Combine Has No Merit

As of Priority Date, different buffers were used to stabilize DNA and blood proteins.



DNA Stabilizing Buffers

- Olson (EX1025)
- Shuber (EX1009)
- Itzkowitz (EX1006)



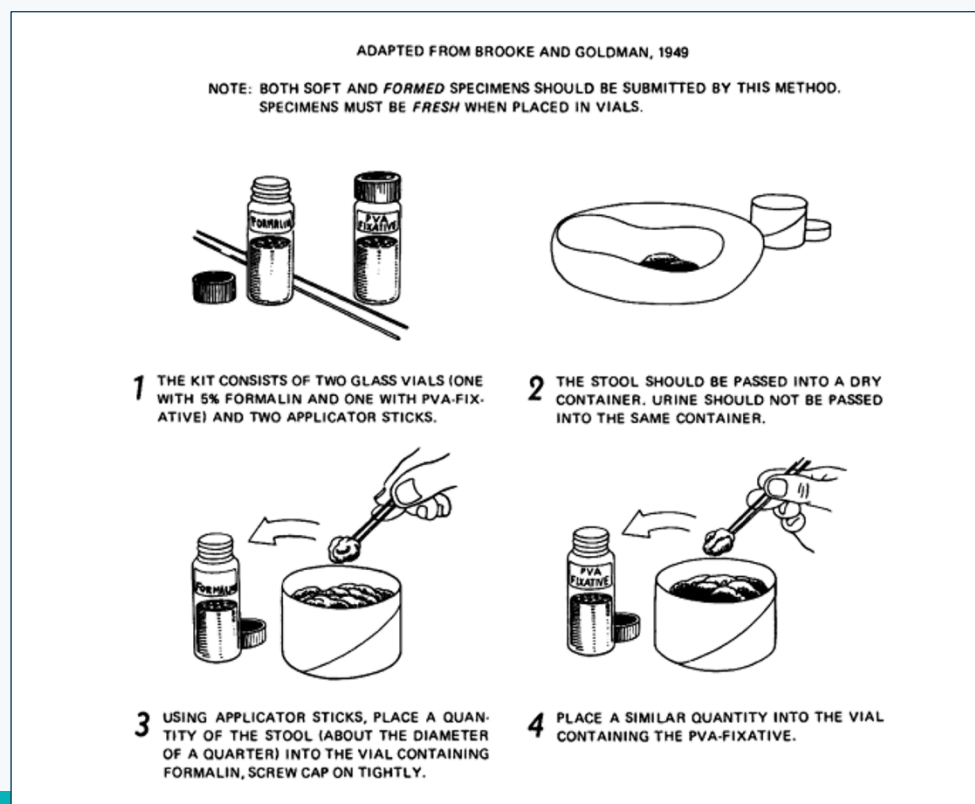
Protein Stabilizing Buffers

- Vilkin (EX1005)
- Grow (EX1042)
- Karl (EX1047)
- Hirata (EX1071)

Reply, at p. 4; EX1002 (Whitney Decl.) ¶¶ 73-81; EX1080 (Whitney 2nd Decl.) ¶¶ 77-81;
EX1025 (Olson), at p. 183; EX1009 (Shuber), at 10:10-15; EX1006 (Itzkowitz), at pp. 111-12;
EX1005 (Vilkin), at pp. 2520-21; EX1042 (Grow), at Abstract; EX1047 (Karl), at p. 1127; EX1071 (Hirata), at p. 1573

Exact's Attack on Motivation to Combine Has No Merit

Partitioning a fecal sample to separately preserve each portion in different stabilizing buffers was well established

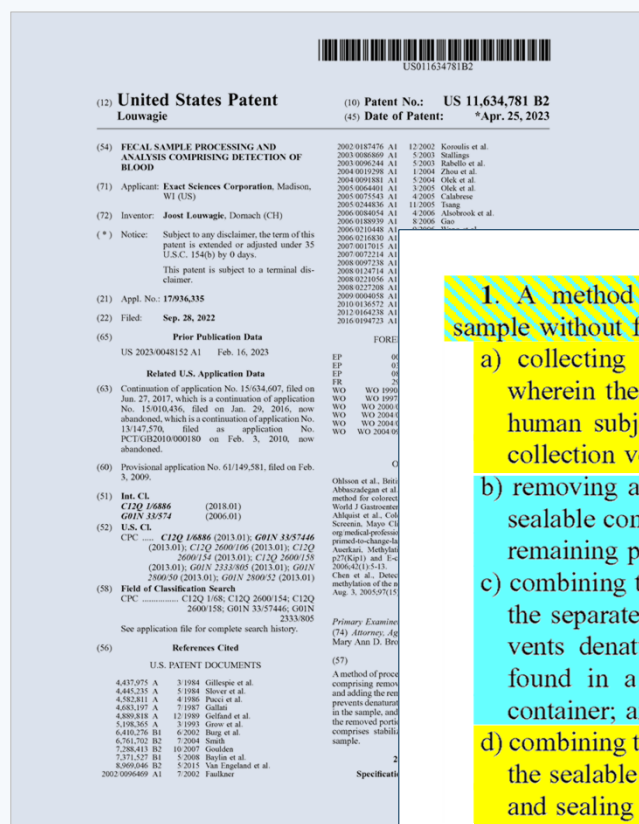


Reply, at p.4;
EX1080 (Whitney 2nd Decl.) ¶ 80;
EX1026 (Melvin), at pp. 7-12

Ground IV: **Shuber and Vilkin**

Claims 1-9, 11, 14-20

Combining Shuber's Fecal DNA Test with Vilkin's iFOBT Results in the Claimed Invention



-  Steps of Shuber Test
-  Steps of Vilkin Test
-  Steps of Both Tests

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.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:

i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and

ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

the pair of portions obtained by the method of claim 1;

b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Petition, at pp. 1, 47-51

Demonstrative Exhibit - Not Evidence

Shuber's Fecal DNA Assay

Shuber

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;

d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:

ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

In any of the aspects of the invention described herein, including the examples, a sample (e.g., a stool sample) may be directly deposited into a container (e.g., a sealable container) that already contains stabilization solution. The amount of solution may be sufficient to cover the sample or a portion thereof. Alternatively, stabilization solution may be added to a container when (e.g., at the same time, immediately after, or within minutes, hours, or a day) a biological sample (e.g., a stool sample) is deposited in the container. The amount of solution added may be sufficient to cover the entire sample or at least a portion thereof (e.g., 10%, 25%, 50%, or more). In one embodiment, an amount of solution sufficient to fill the container may be added. In some embodiment, a container may be between about 25 mls and about 1,000 mls (e.g., between about 50 mls and about 500 mls, between about 100 mls and about 400 mls, or about 250 mls). However, smaller or larger containers may be used. A container with sample and stabilization solution may be sealed for storage/shipping.

In one aspect of the invention, a stabilization solution may be particularly useful when samples are not refrigerated or frozen or when there is a risk that a sample may not be maintained at a sufficiently low temperature to preserve indicia of disease. For example, a stabilization solution may be particularly useful if a sample is obtained at a remote location and mailed or delivered to a testing center. However, stabilization solutions also may be useful to preserve samples that are being processed on-site at a medical center.

Petition, at pp. 19-20, 51-52, 54-57; EX1009 (Shuber), at 10:12-14, 29:8-19

Shuber's Fecal DNA Assay

Shuber

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;

d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.

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

a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:

ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,

c) extracting nucleic acid from the remaining portion of the fecal sample; and

d) testing the nucleic acid for an amount of a human nucleic acid.

Recovery of DNA from stool

The sample preparation methodology used to recover DNA from stool was previously reported (Ahlquist DA, Skoletsky JE, Boynton KA, et al. Colorectal cancer screening by detection of altered human DNA in stool: Feasibility of a multi-target assay panel. Gastroenterology 2000; 119:1219-1227; Whitney D, Skoletsky J, Moore K, et al. Enhanced Retrieval of DNA from Human Fecal Samples Results in Improved Performance of Colorectal Cancer Screening Assay. J Mol. Diagn. 2004; 6 (4), 386-395). Stool aliquots were weighed and combined with Exact buffer A (1:7 (w/v) ratio), and homogenized on an Extractor (Exact Sciences). After homogenization, a 4-g stool equivalent (~32 mls) of each sample was centrifuged to remove all particulate matter. The supernatants were then treated with 20u1 TE buffer (0.01 mol/L Tris [pH 7.4] and 0.001 mol/L EDTA) containing RNase A (2.5 mg/mL), and incubated at 37°C for 1 hour. Total nucleic acid was then precipitated (first adding 1/10 volume 3 mol/L NaAc, then an equal-volume of isopropanol). Genomic DNA was pelleted by centrifugation, the supernatant removed, and the DNA resuspended in TE.

assays. In certain embodiments, methods of the invention are used to preserve a sample for analysis using a nucleic acid integrity assay along with a mutation detection assay (e.g., a multiple mutation panel assay), a hypermethylation assay, or both.

Petition, at pp. 19-20, 51-52, 54-57; EX1009 (Shuber), at 7:19-21, 15:18-16:17

Vilkin's Automated iFOBT

Vilkin

1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:

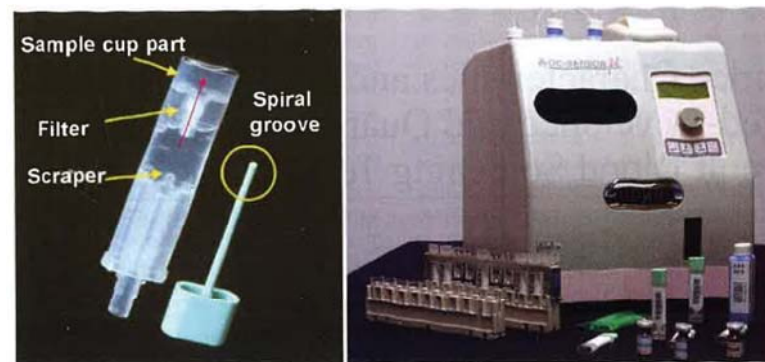
- b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;
- c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and

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

- a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and

- b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;

Petition, at pp. 51-54; EX1005 (Vilkin), at p. 2520



Fecal Sampling

The fecal test sampling device is shaped like a small test tube with the fecal probe inserted into it and sealing it. The probe has a serrated tip, which is poked into the stool and then pushed back into the tube, past a scraper, and through a membrane into the sample cup. These remove most of the excess feces and leave the stool collected (about 10 mg) into the serrations (Fig. 1). The tip is then put in a closed amount of Hb stabilizing buffer. Each sample tube has a unique bar code and before preparing the sample the patient writes his name and date on the tube. The examinee is requested to prepare three consecutive daily samples during the week before colonoscopy examination. Samples are double-closed in zip-lock bags and kept in the refrigerator until returned to the developing laboratory where they are also kept at 4°C until development.

Both Kutzner and Lenhard Experimentally Demonstrate Improved Sensitivity in Combined Assay



Kutzner

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.



Lenhard

more adenomas (31% vs. 8%, $P = .14$). In addition, specificity of the HIC1 assay was higher (97.9% vs. 89.9%, $P = .102$). The combination of both assays resulted in increased detection rates for CRCs (65%; 95% CI, 44%–83%). Although the combined test detected all localized cancers, no increase in the sensitivity for adenoma was seen.

Petition, at pp. 8-9, 49-50;
EX1012 (Kutzner), at p. 40; EX1004 (Lenhard), at p. 147

KSR Int'l Co. v. Teleflex Inc., 550 U.S. 398, 421 (2007)

Demonstrative Exhibit - Not Evidence



39

Grounds II and V: **Ground I or Ground IV and Kanaoka**

Claims 12 and 13

Kanaoka Discloses mRNA as a Known Fecal Biomarker

'781 Patent

12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.

13. The method of claim 12, wherein measuring an amount of RNA expressed from the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).

Nechvetal

to the bacterial constituents of the same human subject. In conclusion, this study suggests that preserving fecal samples at room temperature for a duration of 5 days, in a medium suitable for DNA and RNA analysis is feasible. With development of a suitable collection and shipping device, preservation of fecal samples in RNA later may greatly facilitate self-collection and delivery of stool samples for comprehensive epidemiologic studies of molecular markers for intestinal bacteria, human cancer, and other human factors.

Petition, at pp. 17-19, 43-45, 62-64;
EX1030 (Nechvetal), at p.131;
EX1007 (Kanaoka) ¶¶ 21-25, 67-68, 76

Kanaoka

[0021] Further, the present invention is a tumor marker detecting method for diagnosing colon cancer, comprising the following processes in addition to the process described above:

[0022] b) a process to extract RNA from the obtained sample for extracting RNA;

[0023] c) a process to reverse transcribe the extracted RNA to give cDNA;

[0024] d) a process to amplify the obtained cDNA; and,

[0025] e) a process to detect the amplified cDNA.

* * *

[0067] Among three COX-2 negative colon cancer cases, one was positive in the immunological fecal occult blood test, and 2 were negative.

[0068] COX-2 was detected in 3 among 5 colon cancer cases negative to the immunological fecal occult blood test,

* * *

[0076] Further, since the method of the present invention needs smaller amount of feces for detection and has higher detection sensitivity compared to the detection of the gene mutation of APC, K-ras, or p53, it can save largely the time and effort needed for the detection.

Secondary Considerations

Exact Has Burden To Prove Nexus Between the Asserted Claims and Cologuard's Success



“In order to accord substantial weight to secondary considerations in an obviousness analysis, the evidence of secondary considerations must have a ‘nexus’ to the claims, *i.e.*, there must be ‘a legally and factually sufficient connection’ between the evidence and the patented invention.”

“The patentee bears the burden of showing that a nexus exists.”

Fox Factory, Inc. v. SRAM, LLC,
944 F.3d 1366, 1373 (Fed. Cir. 2019)

Presumption of Nexus Requires Coextensiveness



“[P]resuming nexus is appropriate ‘when the patentee shows that the asserted objective evidence is tied to a specific product and that **product ‘embodies the claimed features, and is coextensive with them.’**”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1373 (Fed. Cir. 2019)

“[T]o meet the coextensiveness requirement, what we do require is that the patentee demonstrate that the **product is essentially the claimed invention.**”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1374 (Fed. Cir. 2019)

Not Coextensive If Product Embodies Claims From Different Patents



“A patent claim is **not coextensive** with a product that includes a ‘critical’ unclaimed feature that is **claimed by a different patent** and that **materially impacts the product’s functionality**.”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1375 (Fed. Cir. 2019)

“Where a product embodies claims from two patents, a **presumption of nexus can be appropriate only if the claims of both patents generally cover the same invention**.”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1377 (Fed. Cir. 2019)

Exact Admits that Cologuard Embodies Over 60 Unrelated Patents Directed to Distinct Inventions

8/9/24, 2:41 PM Patents and Trademarks | Exact Sciences

EXACT SCIENCES

PATENTS & TRADEMARKS

Trademarks

QuARTS, COLOGUARD, EXACT, EXACT SCIENCES, BE SEEN GET SCREENED, and GONE are trademarks of Exact Sciences Corporation. MicroAmp® and Applied Biosystems® are registered trademarks of Life Technologies Corporation (Thermo Scientific). Microlab® is a registered trademark of Hamilton Robotics. ELx808™ is a trademark of BioTek® Instruments Inc. Titer Tops® is a registered trademark of Diversified Biotech. Parafilm® is a registered trademark of Pecheney Plastic Packaging.

Patents

The Cologuard® test and its use are covered by one or more of the following patents associated pending patent applications, or non-U.S. international equivalents: U.S. 8,361,720, 8,673,555, 8,715,937, 8,722,330, 8,916,344, 8,969,046, 8,980,107, 8,993,341, 8,999,176, 9,000,146, 9,024,006, 9,057,098, 9,121,070, 9,121,071, 9,127,318, 9,163,278, 9,169,511, 9,211,112, 9,212,392, 9,290,797, 9,315,853, 9,376,721, 9,399,800, 9,631,228, 9,637,792, 9,657,975, 9,750,482, 9,783,856, 9,803,249, 9,845,491, 9,856,515, 9,982,308, 10,000,817, 10,047,390, 10,093,987, 10,106,844, 10,138,524, 10,144,953, 10,196,676, 10,253,358, 10,265,054, D849,210, 10,435,753, 10,450,614, 10,486,158, 10,519,510, 10,590,489, 10,604,793, 10,626,465, 10,702,250, 10,704,083, 10,808,286, 10,822,639, 10,870,893, 10,889,867, 10,894,256, 11,634,781, 11,845,991, and 11,970,746. This product and its use are also covered by patents pending in the U.S. and around the world.

Dye compounds in the Cologuard® test are sold under license from Biosearch Technologies, Inc. and may be protected by U.S. and world-wide patents or patent applications.

<https://www.exactsciences.com/patents-and-trademarks> Geneoscopy Exhibit 1084, Page 1

Cologuard Website

Patents

The Cologuard® test and its use are covered by one or more of the following patents, associated pending patent applications, or non-U.S. international equivalents: U.S. 8,361,720, 8,673,555, 8,715,937, 8,722,330, 8,916,344, 8,969,046, 8,980,107, 8,993,341, 8,999,176, 9,000,146, 9,024,006, 9,057,098, 9,121,070, 9,121,071, 9,127,318, 9,163,278, 9,169,511, 9,211,112, 9,212,392, 9,290,797, 9,315,853, 9,376,721, 9,399,800, 9,631,228, 9,637,792, 9,657,330, 9,750,482, 9,783,856, 9,803,249, 9,845,491, 9,856,515, 9,982,308, 10,000,817, 10,047,390, 10,093,987, 10,106,844, 10,138,524, 10,144,953, 10,196,676, 10,253,358, 10,265,054, D849,266, 10,435,753, 10,450,614, 10,486,158, 10,519,510, 10,590,489, 10,604,793, 10,626,465, 10,702,250, 10,704,083, 10,808,286, 10,822,639, 10,870,893, 10,889,867, 10,894,256, 11,634,781, 11,845,991, and 11,970,746. This product and its use are also covered by patents pending in the U.S. and around the world.

Reply, at pp. 22-23; EX1084 (Cologuard Website), at p. 1

Exact Admits that Cologuard Embodies Over 60 Unrelated Patents Directed to Distinct Inventions

Unclaimed in '781 Patent	Claimed By U.S. Patent Nos.
Cologuard's DNA stabilizing buffer	6,551,777; 8,722,330
Cologuard's DNA isolation methods	6,268,136; 6,406,857; 8,993,341; 8,999,176; 9,000,146; 9,057,098; 9,163,278; 9,169,511; 9,657,330; 9,631,228; 9,845,491; 9,856,515; 10,047,390; 10,822,639
Cologuard's DNA markers	8,673,555; 8,969,046; 9,121,070; 9,127,318; 9,163,278; 9,169,511; 9,399,800; 9,783,856; 9,803,249; 9,845,491; 10,435,753
Cologuard's methods of preparing DNA	9,315,853; 10,144,953; 10,704,083
Cologuard's methods of testing for methylated DNA	8,916,344; 9,637,792; 10,519,510; 10,590,489; 10,808,286
Cologuard's methods of detecting DNA mutations	8,361,720; 8,715,937; 9,024,006; 9,121,071; 9,212,392; 9,290,797; 9,376,721; 10,000,817; 10,106,844; 10,590,489
Cologuard's control molecules	9,212,392; 10,106,844; 10,138,524; 10,253,358; 10,889,867
Use of stool in CRC screening	5,741,650; 5,952,178; 6,303,304; 6,586,177
Cologuard's collection and storage container	9,211,112; 9,750,482; 10,265,054; 10,486,158; 10,702,250; 10,894,256

Reply, at pp. 22-23; EX1084 (Cologuard Website), at p. 1; EX1080 (Whitney 2nd Decl.) ¶ 105

Cologuard Includes Critical Features Not Claimed by '781 Patent

Device Description

Cologuard utilizes a multi-target approach to detect DNA and hemoglobin markers associated with CRC, as well as pre-malignant colorectal neoplasia (i.e., AA). Three independent categories of biomarkers are targeted and provide an additive association with CRC and pre-malignant colorectal neoplasia.

The first category of biomarkers targets DNA markers associated with CRC, including the NDRG4 and the BMP3 genes. The second category targets DNA markers associated with CRC, including the KRAS gene, the APC gene, and the 7 mutations in Exon 3 of the APC gene. The third category targets DNA markers associated with CRC, including the NDRG4 and the BMP3 genes.

The second category targets DNA markers associated with CRC, including the KRAS gene, the APC gene, and the 7 mutations in Exon 3 of the APC gene. The third category targets DNA markers associated with CRC, including the NDRG4 and the BMP3 genes.

The third category of biomarkers targets DNA markers associated with CRC, including the NDRG4 and the BMP3 genes. The results from the methylation, mutation, and hemoglobin assays are combined in the laboratory analysis to determine a positive or negative reportable result or no result.

Assay Technology

The patient stool samples are analyzed for the presence of DNA markers associated with CRC, including the NDRG4 and the BMP3 genes. The results from the methylation, mutation, and hemoglobin assays are combined in the laboratory analysis to determine a positive or negative reportable result or no result.

Run control samples for DNA and hemoglobin assays and hemoglobin assay are tested along with patient samples to show that the DNA and hemoglobin assays are working properly. Results from the DNA and hemoglobin assays are combined in the laboratory analysis to determine a positive or negative reportable result or no result.

Clinical Study: Multi-Targeted DNA and Hemoglobin Assay for the Detection of Colorectal Cancer and Advanced Adenomatous Polyps

Overview

Cologuard was the subject of a clinical study for the detection of colorectal cancer and advanced adenomatous polyps. The results of the study demonstrated that Cologuard had a sensitivity of 92.3% for the detection of CRC and a specificity of 99.9% for the detection of pre-malignant colorectal neoplasia.



Device Description

Cologuard utilizes a multi-target approach to detect DNA and hemoglobin markers associated with CRC, as well as pre-malignant colorectal neoplasia (i.e., AA). Three independent categories of biomarkers are targeted and provide an additive association with CRC and pre-malignant colorectal neoplasia.

* * *

The third category of biomarker is non-DNA based and detects hemoglobin, which can be associated with colonic bleeding. Results from the methylation, mutation, and hemoglobin assays are combined in the laboratory analysis to determine a positive or negative reportable result or no result.

Cologuard is designed to analyze patients' stool for the presence of 11 molecular markers, including hemoglobin and DNA markers, which may indicate the presence of colorectal cancer or advanced adenomas. Because cellular exfoliation of DNA into stool occurs continuously, Cologuard can detect pre-malignant neoplasia at early onset of abnormality.

Reply, at pp. 22-23; EX1081 (Physician's Brochure), at pp. 3-4; EX1080 (Whitney 2nd Decl.), ¶ 101

Patent Owner's Assertions About Significance of Unclaimed Features in ColoGuard Precludes Presumption of Nexus



“In light of the **patentee’s own assertions about the significance of the unclaimed features**, no reasonable fact finder could conclude that these features are insignificant.”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1375 (Fed. Cir. 2019)

Exact Asserts Significance of Unclaimed Features



Imperiale

DISCUSSION

We compared a multitarget stool DNA test with a commercial FIT among patients at average risk for colorectal cancer. The sensitivity of the DNA test for the detection of both colorectal cancer (92.3%) and advanced precancerous lesions (42.4%) exceeded that of FIT by an absolute difference of nearly 20 percentage points. This difference may be attributed to the DNA marker and algorithm components of the test, since the test performance of the hemoglobin immunoassay component of the DNA test was nearly identical to that of FIT.

Reply, at p. 24; Imperiale (EX2008), at pp. 1294-95

Demonstrative Exhibit - Not Evidence

Exact Asserts Significance of Unclaimed Features

Cologuard Website



INNOVATION SUPPORTS THE COLOGUARD SCIENTIFIC PLATFORM

* * *

STOOL DNA ISOLATION

This proprietary technology proved to be a breakthrough in the development of the multitarget stool DNA test. Our scientists needed to first solve the essential problem of effectively isolating DNA from stool before achieving the goal of developing a noninvasive colon cancer screening test.

DNA BIOMARKER DETECTION

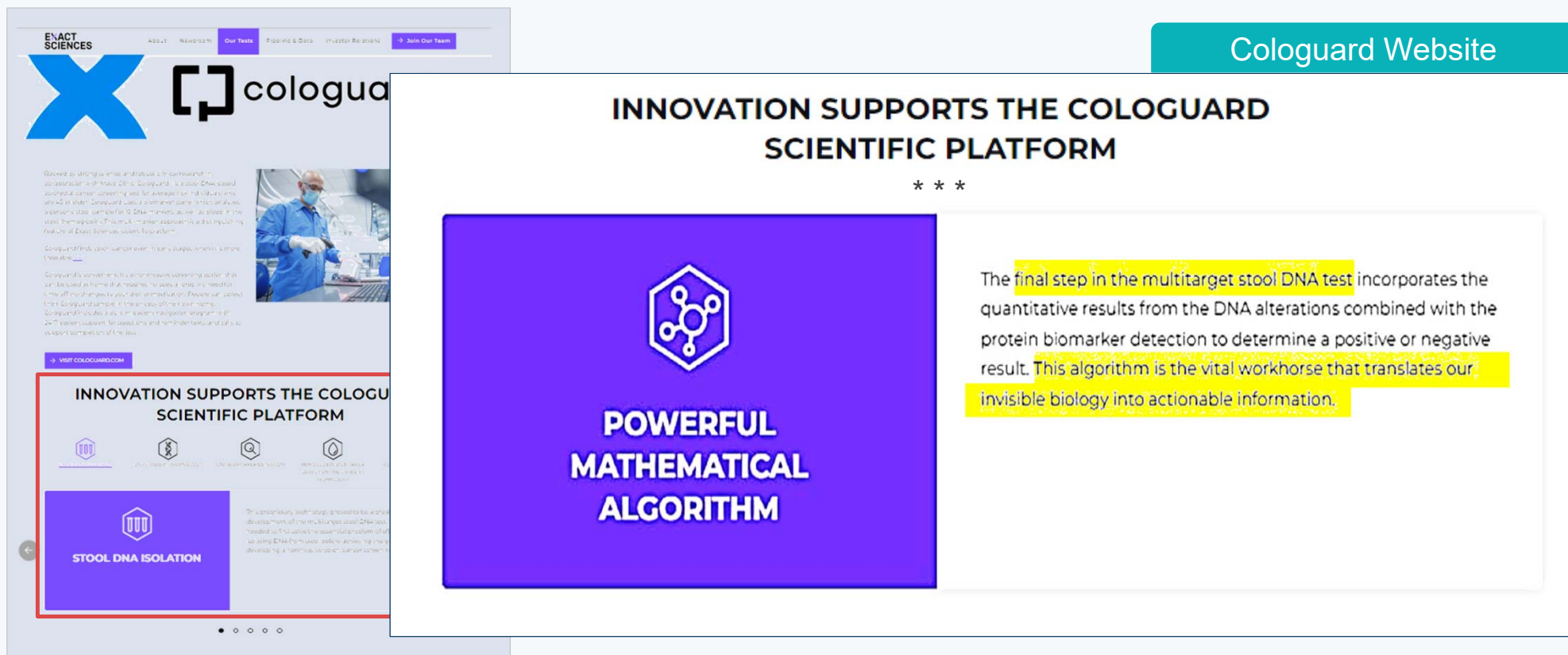
We developed our proprietary QUARTS technology (Quantitative Allele-specific Real-time Target and Signal Amplification) which efficiently amplifies and quantifies two separate methylated DNA markers (NDRC4 and BMP3) along with seven distinct KRAS gene point mutations. The beta actin (ACTB) gene serves as a reference marker for quantitation of the total amount of human DNA in each sample.

EX1088 (Cologuard Website), at pp. 3, 5;
EX1080 (Whitney 2nd Decl.) ¶ 104

Demonstrative Exhibit - Not Evidence

Exact Asserts Significance of Unclaimed Features

Cologuard Website



The screenshot displays the Cologuard website. At the top, the navigation bar includes 'ABOUT', 'NEWS/STORY', 'Our Tests', 'Pricing & Data', 'Investor Relations', and 'Join Our Team'. The main header features the 'EXACT SCIENCES' logo and the 'cologuard' brand name. Below this, a testimonial from a user is shown, accompanied by a photo of a person in a lab coat. The testimonial describes the user's experience with the Cologuard test, highlighting its convenience and accuracy. Below the testimonial, a section titled 'INNOVATION SUPPORTS THE COLOGUARD SCIENTIFIC PLATFORM' is highlighted with a red box. This section features a large blue graphic with a white hexagonal logo containing a stylized 'C' and the text 'POWERFUL MATHEMATICAL ALGORITHM'. To the right of the graphic, a text block states: 'The final step in the multitarget stool DNA test incorporates the quantitative results from the DNA alterations combined with the protein biomarker detection to determine a positive or negative result. This algorithm is the vital workhorse that translates our invisible biology into actionable information.'

EXACT SCIENCES

ABOUT NEWS/STORY Our Tests Pricing & Data Investor Relations Join Our Team

cologuard

Based on strong scientific evidence, the Cologuard test is a non-invasive, at-home stool DNA-based multitarget cancer screening test for average-risk individuals who are at least 45 years old. Cologuard is a comprehensive multitarget stool DNA test that combines a sensitive multitarget stool DNA test with a sensitive multitarget stool DNA test to detect colorectal cancer and precancerous polyps. Cologuard is the only multitarget stool DNA test that is FDA-approved for use in the United States. Cologuard is a non-invasive, at-home stool DNA-based multitarget cancer screening test for average-risk individuals who are at least 45 years old. Cologuard is a comprehensive multitarget stool DNA test that combines a sensitive multitarget stool DNA test with a sensitive multitarget stool DNA test to detect colorectal cancer and precancerous polyps. Cologuard is the only multitarget stool DNA test that is FDA-approved for use in the United States.

INNOVATION SUPPORTS THE COLOGUARD SCIENTIFIC PLATFORM

POWERFUL MATHEMATICAL ALGORITHM

The final step in the multitarget stool DNA test incorporates the quantitative results from the DNA alterations combined with the protein biomarker detection to determine a positive or negative result. This algorithm is the vital workhorse that translates our invisible biology into actionable information.

STOOL DNA ISOLATION

EX1088 (Cologuard Website), at p. 7; EX1080 (Whitney 2nd Decl.) ¶ 104

Demonstrative Exhibit - Not Evidence

Exact Asserts Significance of Unclaimed Features

PATENT
Attorney Docket No.: EXCT-31920/US-3/CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Janelle J. Bruinsma
Serial No.: 14/145,082
Filed: 12/31/2013
Entitled: ISOLATION OF NUCLEIC ACID

AMENDMENT UNDER 37 C.F.R. § 1.312
FINAL OFFICE ACTION MAILED
AND
REQUEST FOR AFTER-FINAL CONSIDERATION

VIA EFS WEB
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF EFS WEB TRANSMISSION UNDER 37 C.F.R. § 1.136(a)(4)
I hereby certify that this correspondence (along with any referred to as herein shown below, being transmitted to the United States Patent and Trademark Office filing system in accordance with 37 C.F.R. § 1.6(a)(4)).
Dated: July 21, 2015 By: Mary Ann D. [Signature]

This communication is an After Final Amendment under the Final Office Communication mailed July 6, 2015, with a request for After-Final Consideration, and is filed with a Request for After-Final Consideration respectfully request entry of this amendment.

The Commissioner is hereby authorized to charge any this application, including fees due under 37 C.F.R. §§ 1.16 and including any required extension of time fees, or credit any over 4302, referencing Attorney Docket No.: EXCT-31920/US-3/CON be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME under § 1.136(a)(3).

Amendments to the Claims are reflected in the Listing of Claims. Remarks begin on page 5.

-1-

Geneoscopy Exhibit 1086, Page 1

Response to Final Office Action, '278 Patent

The references cited in making the rejection, including SHUBER, ZOU, and VAN ENGELAND, demonstrate that there has been a long-felt need for a way to screen for colorectal cancers based on stool DNA testing. However, even in possession of the methods and markers disclosed in the art cited by the Examiner, as stated by VAN ENGELAND, other workers in the field have failed. In contrast, Applicants have been able to meet this need and succeed where others had failed by using a particular combination of markers and methods for extracting the marker DNA, as described in the instant application. The embodiments as claimed herein addressed this long-felt need and succeeded where others had failed, as evidenced by first-ever FDA approval for the product containing these components.

Reply, at pp. 24-25; EX1086 (Response to Final Office Communication, '278 Patent), at p. 9

Exact Has Not Shown Cologuard's Purported Success Is the Direct Result of the Unique Characteristics of the Challenged Claims



“A finding that a presumption of nexus is inappropriate does not end the inquiry into secondary considerations. To the contrary, the patent owner is still afforded an opportunity to prove nexus by showing that the evidence of secondary considerations is the ‘**direct result of the unique characteristics of the claimed invention.**’”

Fox Factory, Inc. v. SRAM, LLC, 944 F.3d 1366, 1373-74 (Fed. Cir. 2019)

- Exact has not proven that “diagnostic accuracy and patient uptake” are unique characteristics of the challenged claims.

Exact Has Not Shown Cologuard's Purported Success Is the Direct Result of the Unique Characteristics of the Challenged Claims

8/9/24, 2:41 PM EXACT SCIENCES PATENTS Trademarks QuARTS, COLOGUARD, EXACT, E... GONE, are trademarks of Exact S... Biosystems® are registered trade... Scientific). Microlab® is a registe... trademark of BioTek® Instrument... Diversified Biotech. Parafilm® is a Patents The Cologuard® test and its use a... associated pending patent applic... 8,361,720; 8,673,555; 8,715,937; 8,72... 8,999,176; 9,000,146; 9,024,006; 9,0... 9,211,112; 9,212,392; 9,290,797; 9,315... 9,750,482; 9,783,856; 9,803,249; 9,8... 10,093,987; 10,106,844; 10,138,524; ... 10,435,753; 10,450,614; 10,486,158; 1... 10,704,083; 10,808,286; 10,822,639... and 11,970,746. This product and i... and around the world. Dye compounds in the Cologuard... Technologies, Inc. and may be pr... https://www.exactsciences.com/patents-and-trademarks	Unclaimed in '781 Patent	Claimed By U.S. Patent Nos.
	Cologuard's DNA stabilizing buffer	6,551,777; 8,722,330
	Cologuard's DNA isolation methods	6,268,136; 6,406,857; 8,993,341; 8,999,176; 9,000,146; 9,057,098; 9,163,278; 9,169,511; 9,657,330; 9,631,228; 9,845,491; 9,856,515; 10,047,390; 10,822,639
	Cologuard's DNA markers	8,673,555; 8,969,046; 9,121,070; 9,127,318; 9,163,278; 9,169,511; 9,399,800; 9,783,856; 9,803,249; 9,845,491; 10,435,753
	Cologuard's methods of preparing DNA	9,315,853; 10,144,953; 10,704,083
	Cologuard's methods of testing for methylated DNA	8,916,344; 9,637,792; 10,519,510; 10,590,489; 10,808,286
	Cologuard's methods of detecting DNA mutations	8,361,720; 8,715,937; 9,024,006; 9,121,071; 9,212,392; 9,290,797; 9,376,721; 10,000,817; 10,106,844; 10,590,489
	Cologuard's control molecules	9,212,392; 10,106,844; 10,138,524; 10,253,358; 10,889,867
	Use of stool in CRC screening	5,741,650; 5,952,178; 6,303,304; 6,586,177
	Cologuard's collection and storage container	9,211,112; 9,750,482; 10,265,054; 10,486,158; 10,702,250; 10,894,256

Reply, at pp. 23-25; EX1084 (Cologuard Website), at p. 1; EX1080 (Whitney 2nd Decl.) ¶ 105

Exact Has Attributed Cologuard's Sensitivity to Unclaimed Features



Imperiale

DISCUSSION

We compared a multitarget stool DNA test with a commercial FIT among patients at average risk for colorectal cancer. The sensitivity of the DNA test for the detection of both colorectal cancer (92.3%) and advanced precancerous lesions (42.4%) exceeded that of FIT by an absolute difference of nearly 20 percentage points. This difference may be attributed to the DNA marker and algorithm components of the test, since the test performance of the hemoglobin immunoassay component of the DNA test was nearly identical to that of FIT.

Reply, at p. 24; EX2008 (Imperiale), at pp. 1294-95

Demonstrative Exhibit - Not Evidence

Exact's Extensive Post-Priority R&D and Marketing Efforts Contributed Significantly to the Patient Uptake of Cologuard

- Even after obtaining FDA approval in 2014, it took Cologuard “many years to gain widespread recognition and adoption.”
- Exact Sciences spent **over \$3.6 billion** dollars on its Cologuard products **between 2009 and 2023**.
- Between **2009 and 2023**, Exact Sciences spent **over \$290 million** in research and development for Cologuard.
- Between **2009 and 2023**, Exact Sciences spent **over \$3 billion** in sales and marketing of Cologuard.

Reply, at p. 26; EX2023 (Declaration of Peter Wood, Exact Sciences' Vice President of Screening Portfolio Strategy) ¶ 13

Exact's Extensive Marketing Efforts Contributed Significantly to Cologuard's Commercial Success

Forbes Article

So Exact adopted a similar strategy, running direct-to-consumer (DTC) marketing tests in five cities shortly after Cologuard's launch. "We ran targeted TV commercials in these five markets and compared them to the five control markets. These markets grew cumulatively 10% per week over the five control markets. After 9 weeks, we were up 100% compared to the control markets." Concludes Conroy: "The Board - and our investors - did not need more proof than that in order for us to really ramp up investment in DTC - \$28 million in 2016."

Reply, at p. 26; EX1087 (Forbes Article Quoting Kevin Conroy, CEO of Exact Sciences), at p. 6

Cologuard is No More Convenient than the Prior Art

Backman Tr.

Q Is the sample collection process for the Cologuard test more convenient than the sample collection process for a FIT test?

A * * *

So from this perspective, I would expect it to be somewhat along the lines -- inconvenience would be similar between Cologuard and FIT. But

Reply, at p. 25; EX1090 (Backman Tr.), at 74:7-9, 75:3-5

Exact Represented to the Patent Office that Cologuard's Success Was Attributable to a Later Invention

PATENT
Attorney Docket No.: EXCT-31920-US-3/CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Janelle J. Bruinsma
Serial No.: 14/145,082
Filed: 12/31/2013
Entitled: ISOLATION OF NUCLEIC ACID

AMENDMENT UNDER 37 C.F.R. § 1.312
FINAL OFFICE ACTION MAILED
AND
REQUEST FOR AFTER-FINAL CONSIDERATION

VIA EFS WEB
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

CERTIFICATE OF EFS WEB TRANSMISSION UNDER 37 C.F.R. § 1.600(c)
I hereby certify that this correspondence (along with any referred to as herein shown below, being transmitted to the United States Patent and Trademark Office filing system in accordance with 37 C.F.R. § 1.600(c)).
Dated: July 21, 2015 By: Mary Ann D. [Signature]

This communication is an After Final Amendment under the Final Office Communication mailed July 6, 2015, with a request for After-Final Consideration, and is filed with a Request for After-Final Consideration respectfully request entry of this amendment.

The Commissioner is hereby authorized to charge any this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17, including any required extension of time fees, or credit any overpayment, 4302, referencing Attorney Docket No.: EXCT-31920-US-3/CON be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME under § 1.136(a)(3).

Amendments to the Claims are reflected in the Listing of Claims. Remarks begin on page 5.

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Geneoscopy Exhibit 1086, Page 1

Response to Final Office Action, '278 Patent

The references cited in making the rejection, including SHUBER, ZOU, and VAN ENGELAND, demonstrate that there has been a long-felt need for a way to screen for colorectal cancers based on stool DNA testing. However, even in possession of the methods and markers disclosed in the art cited by the Examiner, as stated by VAN ENGELAND, other workers in the field have failed. In contrast, Applicants have been able to meet this need and succeed where others had failed by using a particular combination of markers and methods for extracting the marker DNA, as described in the instant application. The embodiments as claimed herein addressed this long-felt need and succeeded where others had failed, as evidenced by first-ever FDA approval for the product containing these components.

Reply, at pp. 24-25; EX1086 (Response to Final Office Communication, '278 Patent), at p. 9

Challenged Claims

'781 Patent, Claim 1

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.

'781 Patent, Claim 2

2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.

'781 Patent, Claim 3

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

- a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and
 - ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer,the pair of portions obtained by the method of claim 1;
- b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;
- c) extracting nucleic acid from the remaining portion of the fecal sample; and
- d) testing the nucleic acid for an amount of a human nucleic acid.

'781 Patent, Claim 4

4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.

'781 Patent, Claim 5

5. The method of claim 4, wherein determining expression from the human gene comprises testing the nucleic acid for the presence of human DNA having an epigenetic modification.

'781 Patent, Claim 6

6. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises measuring an amount of a methylated human DNA.

'781 Patent, Claim 7

7. The method of claim 5, wherein the epigenetic modification comprises aberrant methylation.

'781 Patent, Claim 8

8. The method of claim 7, wherein the aberrant methylation comprises hypermethylation.

'781 Patent, Claim 9

9. The method of claim 5, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.

'781 Patent, Claim 10

10. The method of claim **9**, wherein the gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFP12, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.

'781 Patent, Claim 11

11. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.

'781 Patent, Claim 12

12. The method of claim **4**, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.

'781 Patent, Claim 13

13. The method of claim **12**, wherein measuring an amount of RNA expressed from the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).

'781 Patent, Claim 14

14. The method of claim 3, wherein testing for an amount of a blood protein in the removed portion comprises testing for a concentration of hemoglobin in the removed portion.

'781 Patent, Claim 15

15. The method of claim **14**, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.

'781 Patent, Claim 16

16. The method of claim **14**, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 5 ng/ml.

'781 Patent, Claim 17

17. The method of claim **14**, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 10 ng/ml.

'781 Patent, Claim 18

18. The method of claim **14**, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 20 ng/ml.

'781 Patent, Claim 19

19. The method of claim **14**, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 50 ng/ml.

'781 Patent, Claim 20

20. The method of claim **14**, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 200 ng/ml.