walls come into contact with the membrane, whereby the majority of the specimen of the pasty material does not come into contact with the membrane.

In accordance with a preferred embodiment of the invention, the sampling device comprises an integral disc with its surface being essentially perpendicular to the longitudinal axis of the collecting member. The shape of the disc is such that once the collecting member is introduced into the container, the disc is snugly retained within the inner walls of the container thus stabilizing the position of the collecting member within the container. The disc preferably defines an opening which allows the insertion of a laboratory sampling device, e.g. a pipette, a sampling needle, etc, for withdrawing a portion of the specimen, this without a need to remove the collecting member with the integral disc.

As can be seen, the system of the invention minimize possible contact between a sample and a laboratory technician, which is especially important in cases where the sample may contain hazardous materials or microorganisms.

In accordance with a preferred embodiment of the invention, as already noted above, the pasty material is stool. In accordance with this embodiment, the system may comprise a stool collecting device, which has a collecting member for collecting an excreted stool sample and attachment members for attachment to a toilet seat. The stool collecting device also forms an independent aspect of the invention. The device is typically made of a sheet, e.g. a paper sheet, with the collecting portion and the attachment member being integral, i.e. made of the same sheet. The attachment member may be a sheet portion adapted for wrapping around a toilet seat and are then retained by virtue of being sandwiched between the toilet seat and the upper edges of the toilet bowl. Alternatively, the attachment member may comprise adhesive patches on a bottom face of the attachment member for adherence therethrough to the toilet seat. The adhesive patches are *a priori* protected with the protection being used prior to use and attachment thereof to the toilet seat.

In accordance with an embodiment of the invention, the stool collecting member is a sheet portion suspended between the attachment members. Alternatively, the stool collecting member may have the form of a sack for collecting excreted stool sample. Such a sack may be provided with openings at its bottom to allow a drainage of excreted fluid while retaining the stool.

The stool collecting device is preferably made of a flushable material, e.g. thin paper, and after use may be dropped into the toilet bowl to be flushed thereby.

The invention will now be further illustrated by the following description of a specific embodiment and by the annexed drawings. The illustrated embodiment concerns a system for collecting, storing and transporting stool samples. However, as will no doubt be appreciated by the artisan, this is an example only and the invention pertains also to systems for collecting specimens of pasty material other than a stool.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an exploded view of a system of the invention comprising two containers and a sampling device with two corresponding collecting members;

Fig. 2 shows the system, with the containers being assembled on a base and with the collecting member holding a stool sample;

Fig. 3 shows the system after combining the sampling device with the containers with the collecting member being inserted in the container;

Fig. 4 shows the system after separating between the handle and the collecting members;

Fig. 5 shows the system after closing of the lid, ready for transportation into the laboratory;

Fig. 6 shows a stool collecting device in accordance with the invention;

Figs. 7 and **8** show stool collecting devices in accordance with other embodiments of the invention, attached to a toilet seat.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Reference is first being made to Fig. 1, which is an exploded view showing the various components of the system, generally designated 10. The system 10 comprises two attached containers 12 and 12', a base member 14 with two receptacles 16 and 16' adapted to receive the bottom ends 18 and 18' of containers 12 and 12'; the system further comprises a sampling device 20 having a handle 22 and two collecting members 24 and 24'.

Containers 12 and 12' hold respective media 26 and 26', which may both be the same, but are typically different, e.g. one being a media for preserving bacteria and the other for parasites. Media 26 and 26' are typically semi-solid media, e.g. agar-based, but may also be a lipid, e.g. a culture media. Media 26 and 26' are contained in a portion of the containers sealed by pierceable membranes 28 and 28', the membranes being made of aluminum foil, a laminate of an aluminum foil and a plastic, (e.g. polyethylene) film, etc. Containers 12 and 12' are provided with respective lids 30 and 30' which are linked to the containers by means of linkers 32 and 32'. Sampling device 20 has integral disc members 34 and 34' at the upper end of specimen collecting members 24 and 24'. Disc 34 and 34' have each respective openings 35 and 35', the functions of which will be explained further below. Handle 22 is connected to disc members 34 and 34' by means of breakable neck portions 36 and 36' by small lateral respective movements of the handle and the collecting members connecting necks 36 and 36' break whereby handle 22 and collecting members 24 and 24' are separated.

Collecting members 24 and 24' are elongated and have each a piercing tip 38 and 38' at its bottom end adapted for piercing membranes 28 and 28'. Collecting members 24 and 24' have two lateral walls 40 and 40' which define the stool retaining portion consisting also of planar members 42 and 42' disposed within the lateral walls and carrying a plurality of V-shaped protrusions 44 and 44'. As can be seen, the collecting members

have the largest cross-section at a portion adjacent piercing tips **38** and **38'** thus the hole which is created in the membranes **28** and **28'** allows easy passage therethrough for the remaining portions of the collecting members. Furthermore, lateral walls **40** and **40'** ensure that the stool specimen held on planar members **42** and **42'** is not removed by the membranes **28** and **28'** during passage of collecting members **24** and **24'**.

In order to collect specimens of pasty material, collecting members **24** and **24'** are inserted into a sample of the pasty material, e.g. stool, and thereby specimens are retained on collecting members **24** and **24'**. Sampling device **20** with stool specimens **50** and **50'** retained on collecting members **24** and **24'** is seen in Fig. 2. Also seen in this figure are containers **12** and **12'** assembled on base member **14**.

After collection of the specimens, the sampling device and the containers are combined by first pressing piercing tips **38** and **38'** against membranes **28** and **28'** and then pushing the collecting members until they are accommodated within containers **12** and **12'** with the collecting members being embedded within the medium containing the containers. Fig. 3 shows the sampling device **20** and containers **12** and **12'** immediately after combining the two. As can be seen, the size of disc members **34** and **34'** are such that they are accommodated snugly within the inner walls of the container stabilizing the collecting member in position.

At a next step shown in Fig. 4, the handle **22** is moved naturally thus breaking necks **36** and **36'** leaving the collecting members within containers **12** and **12'**. At a next step shown in Fig. 5, lids **30** and **30'** are used to close containers **12** and **12'** and at this state the containers are shipped to the laboratory. In the laboratory, the lids are opened to a state similar to that shown in Fig. 4, and then a sampling device, e.g. a pipette, a bacteriologic needle, etc., is inserted through openings **35** and **35'**. Thus, sampling of the specimen for laboratory testing does not require removal of the collecting member, as in the case of prior art devices.

A stool collecting device in accordance with an embodiment of the invention is shown in Fig. 6. The device, generally designated **60** is made of a sheet, e.g. paper, particularly thin paper which is flushable in a toilet. The device has a central collecting member, which is a portion of a sheet having the form of a sack, and has two attachment members **64** and **66** which are of a length such that their ends will reach two opposite sides of a toilet seat. The attachment members **64** and **66** have two adhesive patches **68** and **70**, on bottom faces of the device. Adhesive patches **68** and **70** are *a priori* covered by a protective cover (now shown) and prior to use, the cover is removed and then these patches can adhere to the toilet seat. The device is fixed such that stool excreted by a person sitting on the toilet, falls into a sack-like collecting member **62** for later sampling by the sampling device. As can further be seen in Fig. 6, collecting member has at its bottom four openings **72** which are intended to allow a discharge of fluids which may be excreted together with the stool.

Two other embodiments of a stool collecting device are shown in Figs. 7 and 8, fixed to a toilet seat. The stool collecting device **80** shown in Fig. 7, is in principle similar to device **60** shown in Fig. 6 in that it comprises a central sack-like portion **82** and peripheral attachment flaps **84** and **86**. Device **80** is fixed to a toilet seat **88** by means of adhesive patches, similarly as in the case of device **60** in Fig. 6.

Device **90** shown in Fig. 8, comprises a stool collecting portion **92** which is a sheet, e.g. made of paper, suspended between two attachment members **94** and **96** which are two flaps which are wrapped around two opposite sides of the toilet seat **98**, and are fixed by virtue of being clamped between the toilet seat and the underlying toilet bowl (not shown). In addition a device of this embodiment may also comprise adhesive patches at the bottom face members of **94** and **96** to aid in fixing them to the toilet seat.

Having now finalized the description of the specific embodiment, the artisan will no doubt appreciate that the above specific embodiment is

an example only and a myriad of other designs of the container and the sample device as well as the stool collecting device are possible, all being within the scope of the invention as defined in the appended claims.

Patent Owner's Ex. 2005, Page 514

CLAIMS:

1. A system for collecting, storing and transporting of specimens of a pasty material, comprising:

at least one container holding a preservation medium for preserving a chemical entity or living matter to be assayed in the specimen, the container being sealed by a pierceable membrane and having a closure arrangement for sealing the container after the membrane is pierced; and

a sampling device having a handle and at least one elongated specimen collecting member detachably attached thereto, the collecting member being adapted to be received within the container and having a pasty material retaining portion and a piercing tip for piercing the membrane at a lowermost end thereof remote from said handle, for piercing said film.

2. A system according to Claim 1, wherein the medium is of a kind which maintains living matter in a viable condition.

3. A system according to Claim 2, wherein the pasty material is stool.

4. A system according to Claim 1, comprising two containers, one holding a medium for bacteria and another for holding a medium for parasites.

5. A system according to Claim 4, wherein the two containers are attached to one another.

6. A system according to Claim 4 or 5, wherein the sampling device comprises two specimen collecting members.

7. A system according to any one of the preceding claims, wherein said collecting member has a piercing tip for piercing the containers' pierceable membrane and has a pasty material retaining portion.

8. A system according to Claim 7, wherein the pasty material retaining portion has the widest cross-section at a lowermost part thereof, near the piercing tip.

Geneoscopy Exhibit 1070, Page 14

9. A system according to any one of the preceding claims, wherein the sampling device comprises an integral disc in an upper end of the collecting member, with its surface being essentially perpendicular to the longitudinal axis of the collecting member.

10. A system according to Claim 9, wherein the shape of the disc is such that once the collecting member is introduced into the container, the disc is snugly retained within the inner walls of the container.

11. A system according to Claim 10, wherein the disc defines an opening which once the collecting member is placed within the container, allows insertion of a laboratory sampling device into the medium, without a need to remove the collecting member.

12. A stool collecting device which has a collecting member for collecting an excreted stool sample and attachment members for attachment to a toilet seat.

13. A device according to Claim 11, made of a sheet with a stool collecting portion and integral flats for attachment to a toilet seat.

14. A device according to Claim 12, comprising adhesive patches for fixing the device into a toilet seat.

15. A system according to any one of Claims 1-10, comprising a stool collecting device according to any one of Claims 11-13.

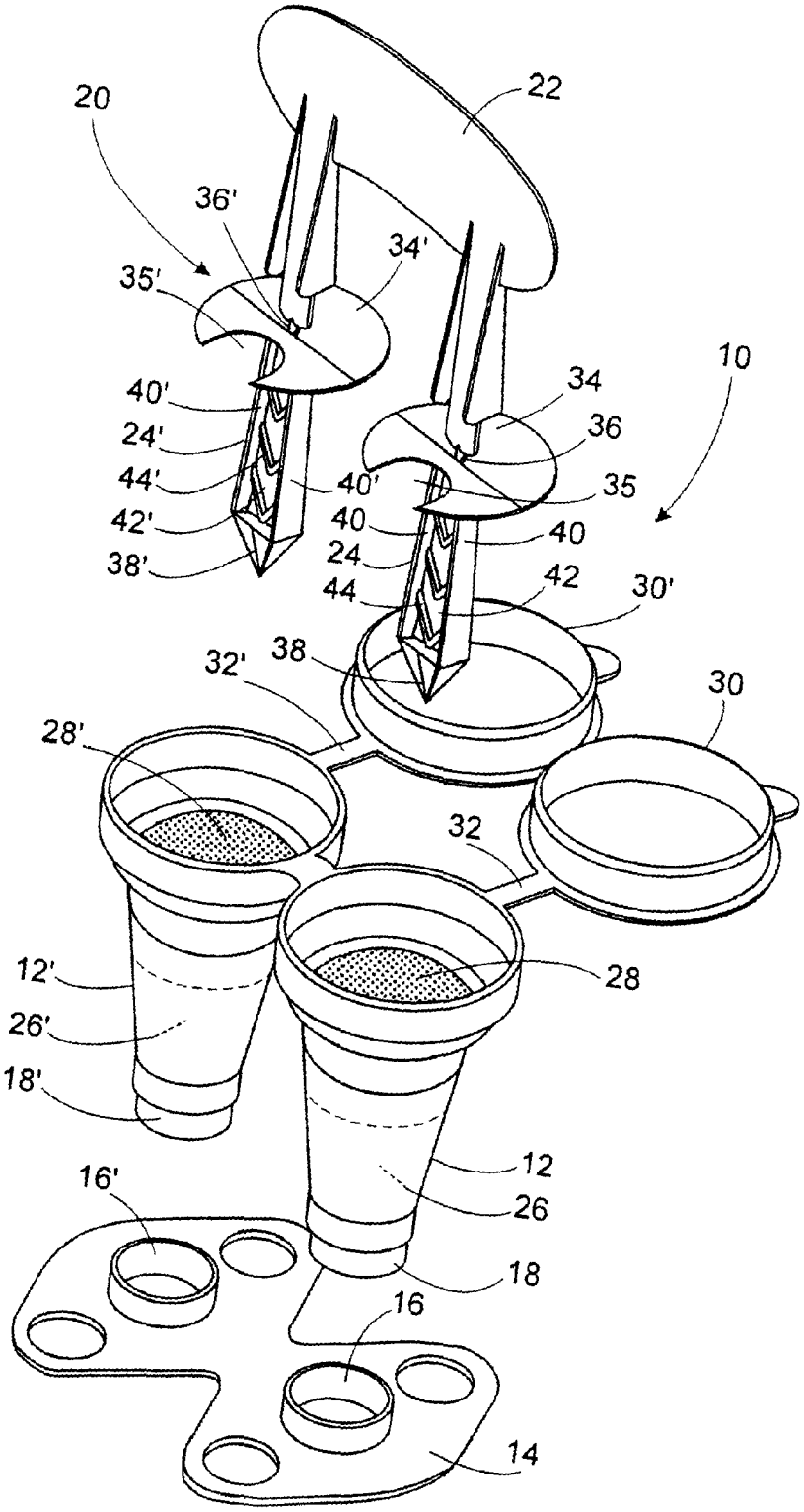

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Fig. 1

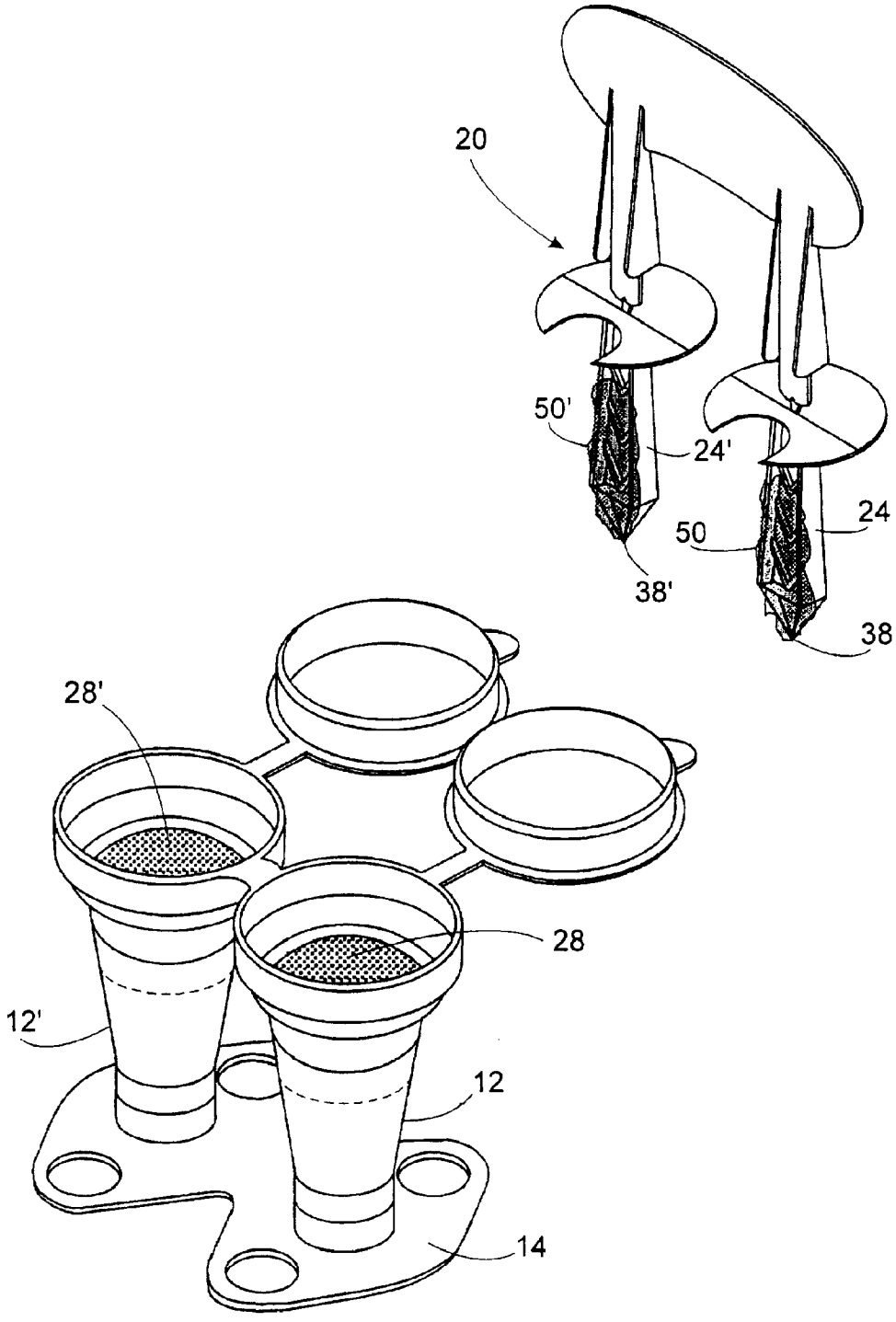


Fig. 2

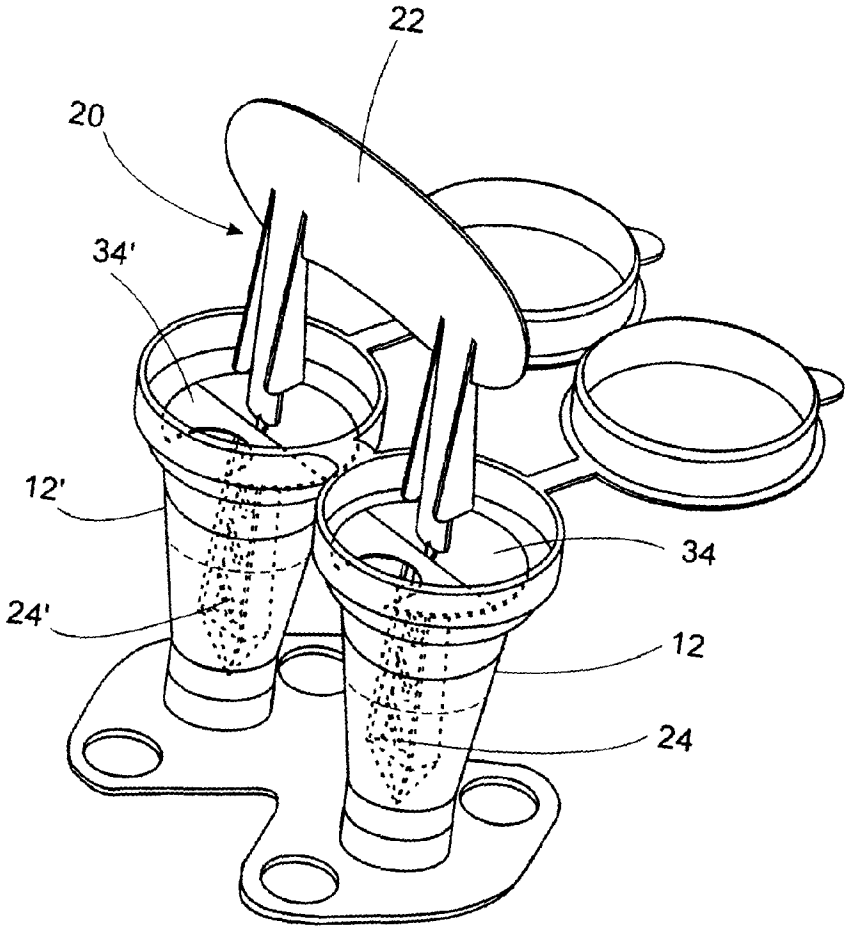


Fig. 3

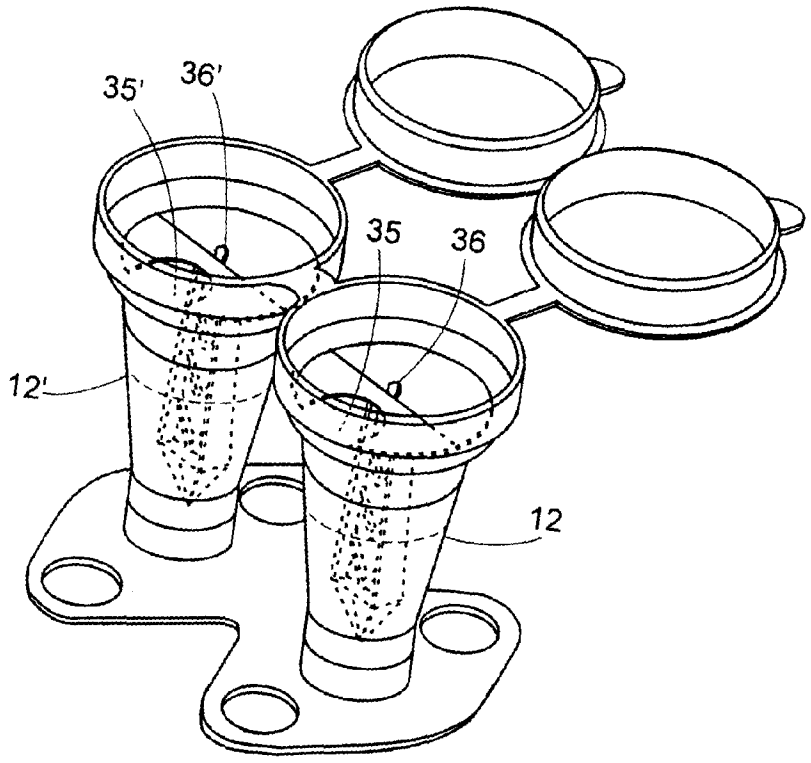


Fig. 4

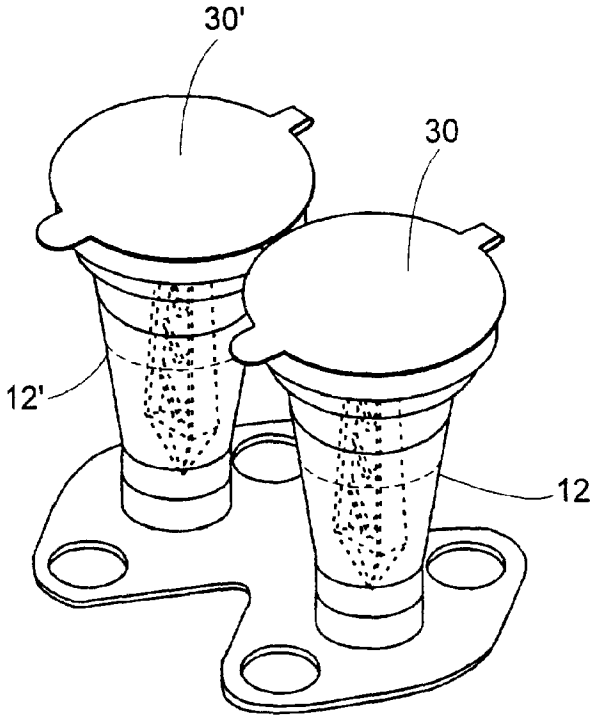


Fig. 5

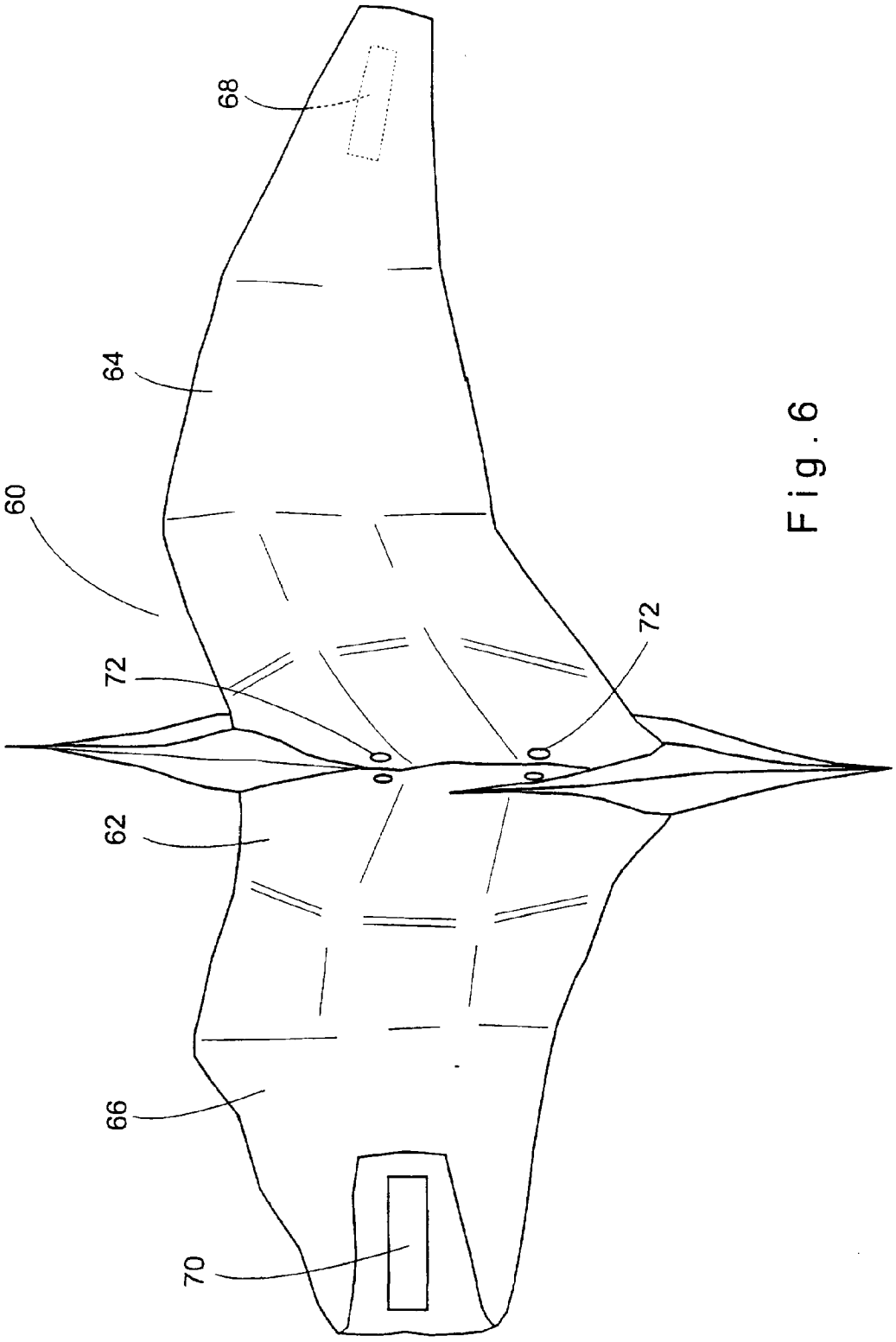


Fig.6

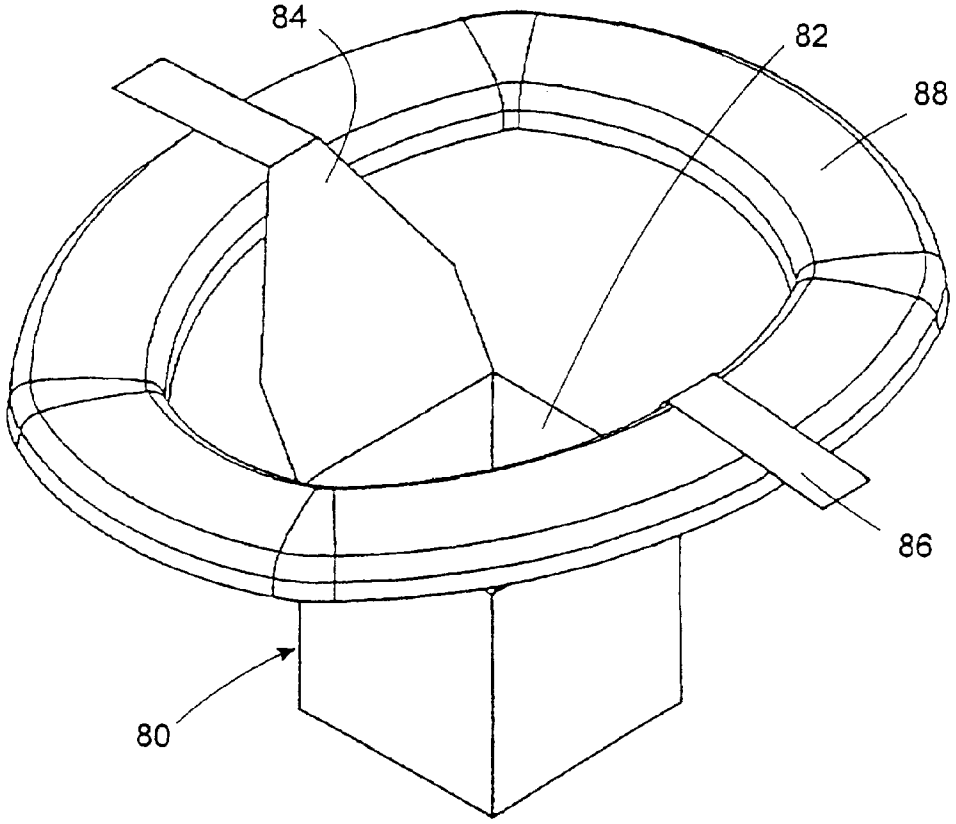


Fig. 7

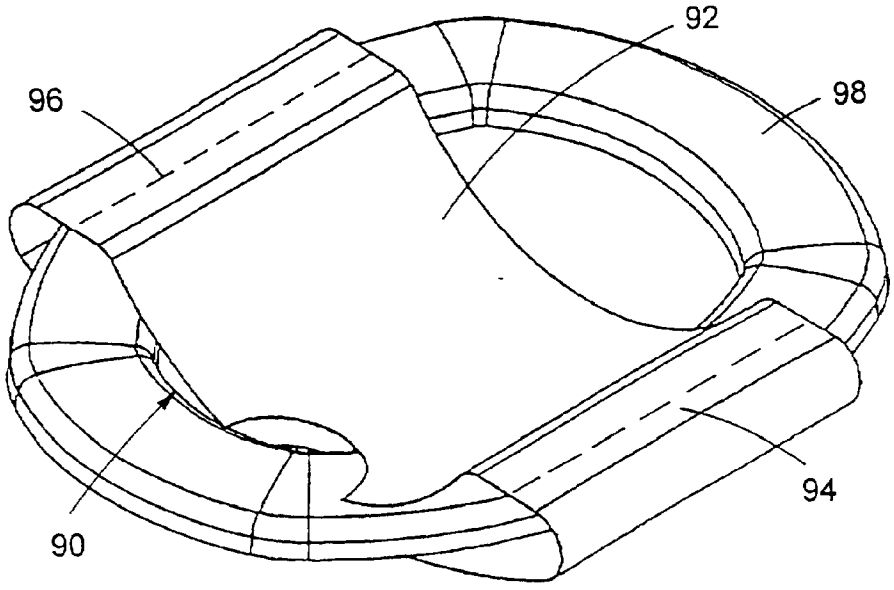


Fig. 8

PART B – FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

173254
Casimir Jones, S.C.
2275 Deming Way, Ste 310
Middleton, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

/Stephanie Filandrinos/	(Typed or printed name)
Stephanie Filandrinos	(Signature)
12/27/2023	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
18/179,945	03/07/2023	LOUWAGIE, Joost	EXCTD-35239.307	2966

TITLE OF INVENTION:

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
CON	LE	$1200	$0.00	$0.00	$1200	02/26/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
WHISENANT, ETHAN C	1634	435-006110

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Casimir Jones, S.C.
2. Mary Ann D. Brow
3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE	(B) RESIDENCE: (CITY and STATE or COUNTRY)
Exact Sciences Corporation	Madison, WI

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☑ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment (*Please first reapply any previously paid fee shown above*):

☑ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 504302

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29. NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27. NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status. NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Mary Ann D. Brow/ Date 12/27/2023

Typed or printed name Mary Ann D. Brow Registration No. 42,363

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

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

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

PATENT
Attorney Docket No.: **EXCTD-35239.307**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	2966
Serial No.:	18/179,945	Group No.:	1634
Filed:	03/07/2023	Examiner:	Ethan C. Whisenant
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

COMMENTS REGARDING EXAMINER'S STATEMENTS ON REASONS FOR ALLOWANCE MAILED 24 NOV 2023 AND CORRECTED NOTICE OF ALLOWABILTY MAILED 20 DEC 2023

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

Applicant is filing the following comments in response to the Examiner's Statement of Reasons for Allowance included with the Notice of Allowance mailed 24 NOV 2023 ("NOA"), and comments made in the Corrected Notice of Allowability mailed 20 DEC 2023 ("CNOA") for the above-referenced application. Applicant agrees with the Examiner's conclusion that claims 1-2 and 4-20 are allowable.

Applicant notes that page 2 of the NOA, second paragraph, includes the text reproduced below:

> ► A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed on 10 JUL 09 in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Claim(s) 27-41 is/are now pending.

(NOA, p2, 2nd para.)

Applicant notes with appreciation that, in the CNOA AT p2, 1st paragraph, the Examiner made the following correction:

> ▶ In the original NOA mailed 24 NOV 2023, the examiner mistakenly referenced an RCE, see 2nd para on p.2. This was a typographical error no RCE was filed in 18/179,945.

(CNOA, p2, 1st para.)

Regarding the NOA, p2, 2nd paragraph reproduced above, Applicant notes that Claims 27-41 are not and have never been pending in the present application and it is Applicant's position that the entirety of this paragraph was included in the NOA in error.

It is further Applicant's position that Examiner's statement in the NOA as originally mailed and as corrected by the Examiner's statement in CNOA, p2, 1st paragraph has no bearing on the allowability, scope, interpretation, or any other aspect of any of allowed claims 1-2 and 4-20 of the instant application. Applicant expressly reserves the right to set forth additional and/or alternative reasons for the patentability of the claims in future applications and/or proceedings.

It is recognized that, in accordance with M.P.E.P. § 1302.14, the Examiner's reasons for allowance need not set forth all of the details as to why the claims are allowed. In the above-referenced application, it is not conceded that the Examiner's stated reasons for allowance are the only reasons for which the claims are allowable. The Examiner's reasons for allowance indicate that particular claim elements are not disclosed or suggested by the prior art of record, yet the claims may be patentable for other reasons as well, including the inventive combination of all of the recited claim elements. Moreover, the Examiner does not assert, and it would not be conceded, that the Examiner's reasons have any bearing on the patentability of claims in any other applications directed to the disclosed subject matter. In addition, each dependent claim stands on its own and may be allowable on its own merits. In particular, each dependent claim may be allowable on the basis of a combination of some of the features recited in the dependent claim and its base claim(s), which combination of features may not include all of the limitations identified in the Examiner's reasons for allowance.

Respectfully submitted,

Dated: December 27, 2023

/Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
608.662.1277

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**12/27/2023 06:07:37 PM Z ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Stephanie Filandrinos
PATENT CENTER #	63767643	FILING DATE	03/07/2023
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-12-27_35239.307_CommentsonAllowance.pdf	3	Post Allowance Communication - Incoming	200 KB
2023-12-27_35239.307_IssueFeeTransmittal.pdf	2	Issue Fee Payment (PTO-85B)	281 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)

2023-12-27_35239.307_CommentsonAllowance.pdf	61B9613270DCDE60A103196F5D8D428262A174ACFEF78865C00300AA7AE4EC84AB2CA5285D6D00864C1DF9FF57F3F349BF9D03ED0EA85AFB9E61904F0D227051
2023-12-27_35239.307_IssueFeeTransmittal.pdf	4A99160B191127D7C87F338B56B8702BF7BD6FFC627F191650693E1B1F56EFE0BEE36F1E8F5990A8A963D49B1581603B99D85C813A0286BB425C60B30816F8F6

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	12/27/2023 06:07:37 PM Z ET	EXCTD-35239.307

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Stephanie Filandrinos
PATENT CENTER #	63767643	AUTHORIZED BY	Mary Ann Brow
CUSTOMER #	173254	FILING DATE	03/07/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Joost Louwagie

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 0638	E2023BQI07525006	Stephanie Filandrinos

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1501	UTILITY ISSUE FEE	1200.00	1	1200.00
			TOTAL AMOUNT:	$1,200.00

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

New Applications Under 35 U.S.C. 111

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage

submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307	2966

173254 7590 12/20/2023

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

EXAMINER	
WHISENANT, ETHAN C	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
12/20/2023	ELECTRONIC

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

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

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

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

Corrected ***Notice of Allowability***	**Application No.** 18/179,945	**Applicant(s)** Louwagie, Joost	
	Examiner Ethan C Whisenant	**Art Unit** 1634	**AIA (FITF) Status** No

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to _____.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☑ The allowed claim(s) is/are 1-2 and 4-20 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

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

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634

Corrected Notice of Allowability

► In the original NOA mailed 24 NOV 2023, the examiner mistakenly referenced an RCE, see 2nd para on p.2 This was a typographical error **no** RCE was filed in 18/179,945.

► Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Wu-Cheng Winston Shen, can be reached at (571) 272-3157.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

UNITED STATES PATENT AND TRADEMARK OFFICE

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

173254 7590 11/24/2023

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

EXAMINER	
WHISENANT, ETHAN C	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 11/24/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307	2966

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	02/26/2024

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

173254 7590 11/24/2023

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

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307	2966

TITLE OF INVENTION: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	02/26/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
WHISENANT, ETHAN C	1634	435-006110

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via the USPTO patent electronic filing system ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (11/23) Approved for use through 03/31/2026 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307	2966

173254 7590 11/24/2023

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

EXAMINER	
WHISENANT, ETHAN C	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 11/24/2023

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

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

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

Notice of Allowability	Application No. 18/179,945	Applicant(s) Louwagie, Joost	
	Examiner Ethan C Whisenant	Art Unit 1634	AIA (FITF) Status No

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 06 OCT 2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☑ The allowed claim(s) is/are 1-2 and 4-20 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

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

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634

NOTICE OF ALLOWABILITY

► **Claim(s) 1-2 and 4-20** as presented in the paper(s) filed 06 OCT 2023 is/are pending in this application.

► A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed on 10 JUL 09 in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Claim(s) 27-41 is/are now pending.

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

REASON(S) FOR ALLOWANCE

► **Claim(s) 1-2, and 4-20** is/are deemed to be allowable in light of the paper(s) filed 06 OCT 2023 and the persuasive argument(s) therein. The Claims have now been renumbered as Claims 1-19 in the same order as presented by the applicant.

Conclusion

C. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Wu-Cheng Winston Shen, can be reached at (571) 272-3157.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	Art Unit
	Ethan C Whisenant	1634

CPC - Searched*		
Symbol	**Date**	**Examiner**
C12Q 1/68 ; 1/6886 ; 2600/106; 154; 158	06/28/2023	ECW
G01N 33/57446 ; 2333/805 ; 2800/50	06/28/2023	ECW
Updated Search	11/16/2023	

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

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

Search Notes		
Search Notes	**Date**	**Examiner**
See the Examiner's Search Notes	06/28/2023	ECW
Updated Search	11/16/2023	ECW

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
C12Q	1/68 ; 1/6886 ; 2600/106; 154; 158	11/16/2023	ECW
G01N	33/57446 ; 2333/805 ; 2800/50	11/16/2023	ECW

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

CPC								
Symbol							**Type**	**Version**
C12Q	/	1	/	6886			F	2013-01-01
G01N	/	33	/	57446			I	2013-01-01
C12Q	/	2600	/	106			A	2013-01-01
C12Q	/	2600	/	154			A	2013-01-01
C12Q	/	2600	/	158			A	2013-01-01
G01N	/	2800	/	50			A	2013-01-01
G01N	/	2800	/	52			A	2013-01-01
G01N	/	2333	/	805			A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	19	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	16 November 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office — Part of Paper No.: 20231116

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

INTERNATIONAL CLASSIFICATION				
CLAIMED				
C12Q1/6886	/	1	/	6886
G01N33/574	/	33	/	574

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	19	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	16 November 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office

Part of Paper No.: 20231116

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

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

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	9	10	18	19										
2	2	10	11	19	20										
	3	11	12												
3	4	12	13												
4	5	13	14												
5	6	14	15												
6	7	15	16												
7	8	16	17												
8	9	17	18												

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	16 November 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office

Part of Paper No.: 20231116

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

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

CLAIMS

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

CLAIM		DATE								
Final	Original	06/28/2023	11/16/2023							
	1	✓	=							
	2	✓	=							
	3	✓	-							
	4	✓	=							
	5	✓	=							
	6	✓	=							
	7	✓	=							
	8	✓	=							
	9	✓	=							
	10	✓	=							
	11	✓	=							
	12	✓	=							
	13	✓	=							
	14	✓	=							
	15	✓	=							
	16	✓	=							
	17	✓	=							
	18	✓	=							
	19	✓	=							
	20	✓	=							

Application Number * 18/179,945 *	Application/Control No. 18/179,945	Applicant(s)/Patent under Reexamination Louwagie, Joost	
	Examiner WHISENANT, ETHAN C	Art Unit 1634	

Document Code - DISQ	Internal Document - DO NOT MAIL

TERMINAL DISCLAIMER	☑ APPROVED	☐ DISAPPROVED
Date Filed: 06 October 2023	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

2/Tds both approved.
/LAWANA R HIXON/

Technology Center: OPLC

Telephone: (571)272-6074

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**10/06/2023 03:19:09 PM ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Mary Ann Brow
PATENT CENTER #	62944184	FILING DATE	03/07/2023
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 5

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
2023-10-06_TermDisclPatent_EXCTD-003USCON4-35239_35239-307.pdf		2	Terminal Disclaimer Filed	237 KB
2023-10-06_TermDisclAppln_EXCTD-003USCON4-35239_35239-307.pdf		2	Terminal Disclaimer Filed	312 KB
2023-10-06_ROA_EXCTD-003USCON4-35239_35239-307.pdf		7	-	194 KB
2023-10-06_ROA_EXCTD-	(1-1)	1	Amendment/Request for	135 KB

003USCON4-35239_35239-307-A....pdf			Reconsideration-After Non-Final Rejection	
2023-10-06_ROA_EXCTD-003USCON4-35239_35239-307-CLM.pdf	(2-5)	4	Claims	175 KB
2023-10-06_ROA_EXCTD-003USCON4-35239_35239-307-REM.pdf	(6-7)	2	Applicant Arguments/Remarks Made in an Amendment	136 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
2023-10-06_TermDisclPatent_EXCTD-003USCON4-35239_35239-307.pdf	64B31EFA8290277FC16AE7DD2DCF9D7712680AC1C4D8B28194A4BA80616217197370072 5E0CAA0C9D929898145DA2191A20FEAC836295790ABE932F00DD751CB
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2023-10-06_ROA_EXCTD-003USCON4-35239_35239-307-A....pdf	72E5212E118944D437E2D79D30EFF197200BEBBA778D999F227349CD6CB07C5CFA0B4D31F1F93E4354B7EB0877802A10B5871816B00A4ACE8501B6E331E9AD06
2023-10-06_ROA_EXCTD-003USCON4-35239_35239-307-CLM.pdf	4B9726E49A040FC769852FA968AF7A2A7423D8CB0CBE48DED3EB09C33346EB63E2DB22FF6C5A43887B065C883531B242BB7B1DE0AFF26883A903563517B4C7FB
2023-10-06_ROA_EXCTD-	7C047E5C0AA843C23666ABE4CD0B7110886FB8275C986C8DB

003USCON4-35239_35239-307-REM.pdf 8C876BE3D4D037BF948EBF0B12AD91B30F393EE3AE0ABDA70AA811E5EA86F256124DB83D37A742D

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PTO/AIA/25 (04-13)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) EXCTD-35239.307

In re Application of: Joost Louwagie

Application No.: 18/179,945

Filed: 7 March 2023

For: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

The applicant, Exact Sciences Corporation, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number 18/179,961, filed, 7 March 2023, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent granted on the pending **reference** application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 42363

/Mary Ann D. Brow/	10/06/2023
Signature	Date
Mary Ann D. Brow	
Typed or printed name	
Attorney	608-662-1277
Title	Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

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PATENT
Attorney Docket No.: **EXCTD-35239.307**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Joost Louwagie	Confirmation:	2966
Serial No.:	18/179,945	Group No.:	1634
Filed:	03/07/2023	Examiner:	Ethan C. Whisenant
Entitled:	**FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD**		

AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION MAILED 07/06/2023

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

This paper is responsive to the Office Action mailed 07/06/2023, with response due by 10/06/2023. Applicant respectfully requests reconsideration in view of the remarks hereinbelow.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: **EXCTD-35239.307**. This paragraph is a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Amendment to the Claims begins on Page 2;

Remarks begin on Page 6.

AMENDMENTS TO THE CLAIMS:

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

1. (original) 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;

   b) in a sealable vessel, combining a first portion of the fecal sample with a stabilizing buffer, and sealing the sealable vessel; and

   c) in a sealable container, combining a second portion of the fecal sample with a solution that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container.

2. (original) The method of claim 1, further comprising delivering the sealable vessel containing the first portion of the fecal sample and the stabilizing buffer and the sealable container containing the second portion of the fecal sample and the solution to a medical diagnostics laboratory.

3. (canceled)

4. (original) 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 vessel containing a first portion of a fecal sample and a stabilizing buffer; and

      ii) a sealed sealable container containing a second portion of a fecal sample and a solution that prevents denaturation or degradation of blood proteins found in a fecal sample,

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

b) extracting nucleic acid from the first portion of the fecal sample;

c) testing nucleic acid extracted from the first portion of the fecal sample for an amount of a human nucleic acid; and

d) testing the second portion of the fecal sample for an amount of a blood protein present in the second portion of the fecal sample.

5. (original) The method of claim 4, wherein testing the nucleic acid comprises determining expression from a human gene.

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

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

8. (original) The method of claim 6, wherein the epigenetic modification comprises aberrant methylation.

9. (original) The method of claim 8, wherein the aberrant methylation comprises hypermethylation.

10. (original) The method of claim 6, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.

11. (original) The method of claim 10, wherein the gene is selected from the group consisting of *PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2,* and *CDO1*.

12. (original) The method of claim 6, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil.

13. (original) The method of claim 5, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the human gene.

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

15. (original) The method of claim 4, wherein testing for an amount of a blood protein present in the second portion comprises testing for a concentration of hemoglobin in the second portion, wherein a concentration of hemoglobin is indicative of a presence of blood in the fecal sample.

16. (original) The method of claim 15, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.

17. (original) The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 5 ng/ml.

18. (original) The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 10 ng/ml.

19. (original) The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 20 ng/ml.

20. (original) The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 50 ng/ml.

REMARKS

Information Disclosure Statements

Claims 1-20 are pending in the present application. Applicant notes with appreciation that the Examiner has considered the references disclosed by the Applicant in the Information Disclosure Statements filed in this matter on 03/07/2023 and 06/08/2023.

Claim Amendments

For business reasons and without acquiescing to Examiner arguments, and reserving the right to prosecute the original or similar claims in one or more future applications, Applicant herein cancels claim 3 without prejudice.

Office Action

In an Office Action mailed 07/06/2023, the Examiner provisionally rejected all claims on the grounds of non-statutory obviousness-type double patenting over claims 1-19 of co-pending Application Ser No. 18/179,961.

The Examiner additionally rejects Claims 1-20 over claims 1-20 of U.S. Patent No. 11,634,781.

Solely in the interest of expediting prosecution, Applicant herewith files terminal disclaimers over Application Ser. No. 18/179,961 and US Patent No. 11,634,781 to obviate the present rejections. Applicant notes that the filing of a terminal disclaimer to obviate a rejection based on non-statutory double patenting is not an admission of the propriety of the rejection.

CONCLUSION

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

Dated: October 6, 2023

/Mary Ann D. Brow/

Mary Ann D. Brow
Registration No. 42,363

CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
608.662.1277

PTO/AIA/26 (04-14)
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TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) EXCTD-35239.307

In re Application of: Joost Louwagie

Application No.: 18/179,945

Filed: 7 March 2023

For: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

The applicant, Exact Sciences Corporation, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 11,634,781 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 42363

/Mary Ann D. Brow/ — Signature
10/06/2023 — Date

Mary Ann D. Brow — Typed or printed name

Attorney — Title
608-662-1277 — Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) included.

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A Federal agency may not conduct or sponsor, and a person is not required to respond to, nor shall a person be subject to a penalty for failure to comply with an information collection subject to the requirements of the Paperwork Reduction Act of 1995, unless the information collection has a currently valid OMB Control Number. The OMB Control Number for this information collection is 0651-0031. Public burden for this form is estimated to average 12 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the information collection. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden to the Chief Administrative Officer, United States Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450 or email InformationCollection@uspto.gov. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **If filing this completed form by mail, send to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013). https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to: 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law; 2) a Federal, state, local, or international agency, in response to its request; 3) a contractor of the USPTO having need for the information in order to perform a contract; 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record; 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record; 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations; 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals; 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)); 9) the Office of Personnel Management (OPM) for personnel research purposes; and 9) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Additional Uses

Additional USPTO uses of the information in this record may include disclosure to: 1) the International Bureau of the World Intellectual Property Organization, if the record is related to an international application filed under the Patent Cooperation Treaty; 2) the public i) after publication of the application pursuant to 35 U.S.C. 122(b), ii) after issuance of a patent pursuant to 35 U.S.C. 151, iii) if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections, or an issued patent, or iv) without publication of the application or patent under the specific circumstances provided for by 37 CFR 1.14(a)(1)(v)-(vii); and/or 3) the National Archives and Records Administration, for inspection of records.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**10/06/2023 03:19:09 PM ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Mary Ann Brow
PATENT CENTER #	62944184	AUTHORIZED BY	Mary Ann Brow
CUSTOMER #	173254	FILING DATE	03/07/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Joost Louwagie

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 0638	**E202306F19279874**	**Stephanie Filandrinos**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1814	STATUTORY DISCLAIMER, INCLUDING TERMINAL DISCLAIMER	170.00	2	340.00
			TOTAL AMOUNT:	**$340.00**

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307

CONFIRMATION NO. 2966

173254
CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

PUBLICATION NOTICE

OC000000062913983

Date Mailed: 10/05/2023

Title:FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Publication No.US-2023-0313317-A1
Publication Date:10/05/2023

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/179,945	03/07/2023	Joost Louwagie	EXCTD-35239.307	2966

173254 7590 07/06/2023

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

EXAMINER	
WHISENANT, ETHAN C	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
07/06/2023	ELECTRONIC

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

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

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

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

Office Action Summary	Application No. 18/179,945	Applicant(s) Louwagie, Joost	
	Examiner Ethan C Whisenant	Art Unit 1634	AIA (FITF) Status No

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

Period for Reply

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

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

Status

1)☑ Responsive to communication(s) filed on 07 MAR 2023.

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

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

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

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

Disposition of Claims*

5) ☑ Claim(s) 1-20 is/are pending in the application.

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

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-20 is/are rejected.

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

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

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

Application Papers

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

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

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

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

Priority under 35 U.S.C. § 119

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

Certified copies:

a)☑ All b)☐ Some** c)☐ None of the:

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

2.☑ Certified copies of the priority documents have been received in Application No. 13147570.

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

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

Attachment(s)

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

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 07 MAR 2023.

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

4) ☐ Other: _____.

Detailed Action

► **Claim(s) 1-20** as presented in the paper(s) filed 07 MAR 2023 is/are pending.

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

Non-Statutory Obviousness-type Double Patenting

► The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969). A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b). A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should

be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Non-Statutory Obviousness-type Double Patenting Rejections

► **Claim(s) 1-20** is/are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-19 of copending Application No.18/179,961. Although the conflicting claims are not identical, they are not patentably distinct**. This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented**.

► **Claim(s) 1-20** is/are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-20 of U.S. Patent No. 11,634,781 (hereinafter "US -781'").

Although the conflicting claims are not identical, they are not patentably distinct from each other. For example, Claim 1 of US-781 teach a method of processing a freshly-collected fecal sample without freezing that comprises collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject; in a sealable vessel, combining a first portion of the fecal sample with a stabilizing buffer, and sealing the sealable vessel; and in a sealable container, combining a second portion of the fecal sample with a solution that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container.

Prior Art

► The Claims are allowable over the prior art of record because the prior art of record fails to teach dividing a feces/stool sample collected at home into at least two portions. One portion of which is combined with a 1st stabilizing buffer (i.e. nucleic acid stabilizing buffer) while the second portion is combined with a solution that prevents denaturation or degradation of blood proteins found in the fecal sample. The closest prior art is considered to be Lapidus et al. [US 5,952,178 – hereinafter "Lapidus"] - cited by applicant. However, Lapidus does not teach or reasonably suggest dividing a feces sample collected at home into at least two portions. One portion of which is combined with a 1st stabilizing buffer (i.e. nucleic acid stabilizing buffer) while the second portion is combined with a solution that prevents denaturation or degradation of blood proteins found in the fecal sample. The teaching of Lapidus alone or in combination with the other prior art of record would have suggested to PHOSITA to place the a stool, or a portion thereof, collected at home into a sealable container and to combine said stool or portion thereof with a stabilizing buffer which stabilizing buffer stabilizes both nucleic acids and proteins found within the stool sample or portion thereof. The claims clearly require the division of the stool into two separate and distinct samples. A first for nucleic acid analysis and a second for protein analysis. The prior art of record fails to teach this feature.

Conclusion

C. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30 am -5:30 pm EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Wu-Cheng Winston Shen, can be reached at (571) 272-3157.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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

/ETHAN C WHISENANT/
Primary Examiner, Art Unit 1634
ethan.whisenant@uspto.gov

EXAMINER SEARCH NOTES

28 JUN 2023 - ECW

Databases searched: All available via PE2E SEARCH
CAplus, Medline and BIOSIS via STNext; and **Google Scholar** (note the search terms used below)

Reviewed the parent(s), if any, and any search(es) performed therein : see the BIB data sheet

Reviewed, the search(es), if any, performed by prior examiners including any international examiners. Reviewed the Search Report and Written opinion in PCT/GB2010/000180

Planned Search

Search terms:

All Inventor(s) e.g. Louwagie?/au
Feces or faeces or stool
Process$
Collect$
divid$ or partion$ or portion$ or separat$
sealable (vessel$ or container$)
blood proteins
colorectal cancer or CRC

► See the Examiner's PE2E SEARCH notes/strategy in IFW

Notice of References Cited	Application/Control No. 18/179,945	Applicant(s)/Patent Under Reexamination Louwagie, Joost	
	Examiner Ethan C Whisenant	Art Unit 1634	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	JP-4039590-B2	01-2008	JP	Yoshizawa et al.	
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Nechvatal et al. Journal of Microbiological Methods 72: 124-132 (Year: 2008)
	V	
	W	
	X	

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

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	**Art Unit**
	Ethan C Whisenant	1634

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

CLAIMS

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

CLAIM		DATE								
Final	Original	06/28/2023								
	1	✓								
	2	✓								
	3	✓								
	4	✓								
	5	✓								
	6	✓								
	7	✓								
	8	✓								
	9	✓								
	10	✓								
	11	✓								
	12	✓								
	13	✓								
	14	✓								
	15	✓								
	16	✓								
	17	✓								
	18	✓								
	19	✓								
	20	✓								

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/179,945	Louwagie, Joost
	Examiner	Art Unit
	Ethan C Whisenant	1634

CPC - Searched*		
Symbol	Date	Examiner
C12Q 1/68 ; 1/6886 ; 2600/106; 154; 158	06/28/2023	ECW
G01N 33/57446 ; 2333/805 ; 2800/50	06/28/2023	ECW

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

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

Search Notes		
Search Notes	Date	Examiner
See the Examiner's Search Notes	06/28/2023	ECW

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

/ETHAN C WHISENANT/ Primary Examiner, Art Unit 1634	

Bibliographic Data

Application No: 18/179,945

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☑ No ☐ Met After Allowance

Verified and Acknowledged: /ETHAN C WHISENANT/ Examiner's Signature | Initials

Title: FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
03/07/2023	435	1634	EXCTD-35239.307
RULE			

APPLICANTS

Exact Sciences Corporation, Madison, WI, UNITED STATES

INVENTORS

Joost Louwagie, Dornach, SWITZERLAND

CONTINUING DATA

This application is a CON of 17936335 09/28/2022 PAT 11634781

17936335 is a CON of 15634607 06/27/2017

15634607 is a CON of 15010436 01/29/2016ABN

15010436 is a CON of 13147570 03/12/2012ABN

13147570 is a 371 of PCT/GB2010/000180 02/03/2010

PCT/GB2010/000180 has PRO of 61149581 02/03/2009 *

(*) Data provided by applicant is not consistent with PTO record

FOREIGN APPLICATIONS

PCT/GB2010/000180 02/03/2010

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05/19/2023

STATE OR COUNTRY

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2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

FILING FEE RECEIVED

$6,160

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	18/179,945
				Filing Date	03/07/2023
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	1	of	2	Attorney Docket Number	EXCTD-35239.307

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4859610	1989-08-22	MAGGIO	
		5137806	1992-08-11	LEMAISTRE et al.	
		5196167	1993-03-23	GUADAGNO et al.	
		5527676	1996-06-18	VOGELSTEIN et al.	
		5741650	1998-04-21	LAPIDUS et al.	
		5952178	1999-09-14	LAPIDUS et al.	
		11634781	2023-04-25	LOUWAGIE	

U.S. PUBLISHED PATENT APPLICATIONS

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

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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] (if known)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 1366715	2003-12-03	SENTINEL CH. S.r.L		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		

NONPATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]

Examiner Signature	/ETHAN C WHISENANT/	Date Considered	06/28/2023

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NONPATENT LITERATURE DOCUMENTS			
		AHLQUIST et al., Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia. Ann Intern Med. 2008 Oct 7;149(7):441-W81	
		HOEPFFNER et al., Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study. Aliment Pharmacol Ther. 2006 Jan 1;23(1):145-54	
		LEUNG et al., Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of Patients with Colorectal Cancer or Polyps. Am J Gastroenterol 2007;102:1070-6	
		OLSON et al., DNA Stabilization is Critical for Maximizing Performance of Fecal DNA-Based Colorectal Cancer Tests. Diagn Mol Pathol 2005;14:183-191	
		Request for Ex Parte Reexamination of U.S. Patent 11,634,781, filed May 22, 2023, 286 pages	
		Declaration of Mr. Anthony P. Shuber, MS, Reexam Application 90/015,237, filed May 22, 2023, 63 pages	

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Sheet	3	of	2	Attorney Docket Number	EXCTD-35239.307

CERTIFICATION STATEMENT

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☐ See attached certification statement.

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

☒ A certification statement is not submitted herewith.

☐ Documents not provided herewith have been previously cited in parent U.S. patent application number ____ filed on_____. In compliance with 37 C.F.R. § 1.98(d), Applicants have not included copies of these documents.

SIGNATURE

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Signature	/Mary Ann D. Brow/	Date (YYYY-MM-DD)	2023-06-08
Name/Print	Mary Ann D. Brow	Registration Number	42363

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[8] Applicant is to place a check mark here if English language Translation is attached.
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				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	1	of	5	Attorney Docket Number	EXCTD-35239.307

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4437975	1984-03-20	GILLESPIE et al.	
		4445235	1984-05-01	SLOVER et al.	
		4582811	1986-04-15	PUCCI et al.	
		4683197	1987-07-28	GALLATI	
		4889818	1989-12-26	GELFAND et al.	
		5198365	1993-03-30	GROW et al.	
		6410276	2002-06-25	BURG et al.	
		6761702	2004-07-13	SMITH	
		7288413	2007-10-30	GOULDEN	
		7371527	2008-05-13	BAYLIN et al.	
		8969046	2015-05-03	VAN ENGELAND et al.	

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20020187476	2002-12-12	KOROULIS et al.	
		20020096469	2002-07-25	FAULKNER	
		20030086869	2003-05-08	STALLINGS	
		20030096244	2003-05-22	RABELLO et al.	
		20040019298	2004-01-29	ZHOU et al.	
		20040091881	2004-05-13	OLEK et al.	
		20050064401	2005-03-24	OLEK et al.	
		20050075543	2005-04-07	CALABRESE	
		20050244836	2005-11-03	TSANG	
		20060084054	2006-04-20	ALSOBROOK et al.	
		20060188939	2006-08-24	GAO	
		20060210448	2006-09-21	WANG et al.	

Examiner Signature	/ETHAN C WHISENANT/	Date Considered	06/28/2023

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U.S. PUBLISHED PATENT APPLICATIONS					
		20060216830	2006-09-28	KIKUIRI	
		20070017015	2007-01-25	FINELL	
		20070072214	2007-03-29	GARVIN	
		20080097238	2008-04-24	LOKTIONOV et al.	
		20080124714	2008-05-29	SHUBER et al.	
		20080221056	2008-09-11	BAYLIN et al.	
		20080227208	2008-09-18	YEE et al.	
		20090004058	2009-01-01	LIANG et al.	
		20100136572	2010-06-03	ATAMAN-ONAL et al.	
		20120164238	2012-06-28	LOUWAGIE	
		20160194723	2016-07-07	LOUWAGIE	
		20230048152	2023-02-16	LOUWAGIE	

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		EP 0032782	1981-07-29	KOMMANDIITTIYHTIO FINNPIPETTE		
		EP 0308227	1989-03-22	SMITHKLINE DIAGNOSTICS, INC.		
		EP 0817968	1998-01-14	EXACT SCIENCES CORPORATION		
		FR 2919065	2009-01-23	BIOMERIEUX		
		WO 1990/06995	1990-06-28	SISKA DIAGNOSITCS, INC.		
		WO 1997/46705	1997-12-11	THE JOHNS HOPKINS SCHOOL OF MEDICINE		
		WO 2000/050640	2000-08-31	EXACT LABORATORIES, INC.		
		WO 2004/067726	2004-08-12	KECK GRADUATE INSTITUTE		

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FOREIGN PATENT DOCUMENTS						
		WO 2004/083399	2004-09-30	THE JOHNS HOPKINS UNIVERSITY.		
		WO 2004/0927209	2004-10-28	KIETURAKIS & KARCZEWSKI		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		
		WO 2005/017207	2005-02-24	CASE WESTERN RESERVE UNIVERSITY		
		WO 2006/113671	2006-10-26	ONCOMETHYLOME SCIENCES SA		
		WO 2008/010975	2008-01-24	THE JOHNS HOPKINS UNIVERSITY		
		WO 2008/084219	2008-07-17	ONCOMETHYLOME SCIENCES SA		
		WO 2008/100913	2008-08-21	THE JOHNS HOPKINS UNIVERSITY		
		WO 2008/102002	2008-08-28	RIKSHOSPITALET RADIUMHOSPITALE		

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		ABBASZADEGAN et al., Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran. World J Gastroenterol. 2007 Mar 14;13(10):1528-33	
		AHLQUIST et al., Cologuard Primed to Change Landscape of CRC Screenin", Mayo Clinic Clinical Updates, http://www.mayoclinic.org/medical-professionals/clinical-updates/digestivediseases/ cologuard-primed-to-change-landscape-of-crc-screening, Pages 1-4, 12/03/2014.	
		AUERKARI, Methylation of tumor suppressor genes p16(INK4a), p27(Kip1) and E-cadherin in carcinogenesis. Oral Oncol. 2006 Jan;42(1):5-13	
		CHEN et al., Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. J Natl Cancer Inst. 2005 Aug 3;97(15):1124-32	
		CONROY et al., Exact Sciences Completes 40,000 Cologuard Tests During First Quarter 2016, Exact Sciences Latest News, Pages 1-10, 05/03/2016.	

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NONPATENT LITERATURE DOCUMENTS			
		EXACT SCIENCES, Cologuard(TM) sDNA-based Colorectal Cancer Screening Test - Instructions for Use, Pages 1-71, 2013.	
		HAMMER, Human hybirds. Sci Am. 2013 May;308(5):66-71	
		ITZKOWITZ et al., A simplified, noninvasive stool DNA test for colorectal cancer detection. Am J Gastroenterol. 2008 Nov;103(11):2862-70	
		KARL et al., Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. Clin Gastroenterol Hepatol. 2008 Oct;6(10):1122-8	
		KIM et al., Noninvasive Molecular Biomarkers for the Detection of Colorectal Cancer. BMB Rep. 2008 Oct 31;41(10):685-92	
		KUTZNER et al., Non-invasive detection of colorectal tumours by the combined application of molecular diagnosis and the faecal occult blood test. Cancer Lett. 2005 Nov 8;229(1):33-41	
		LAIRD, The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003 Apr;3(4):253-66	
		LENHARD et al., Analysis of promoter methylation in stool: a novel method for the detection of colorectal cancer. Clin Gastroenterol Hepatol. 2005 Feb;3(2):142-9	
		LEVI et al., A quantitative immunochemical faecal occult blood test is more efficient for detecting significant colorectal neoplasia than a sensitive guaiac test. Aliment Pharmacol Ther. 2006 May 1;23(9):1359-64	
		OHLSSON et al., Biomarker selection for detection of occult tumour cells in lymph nodes of colorectal cancer patients using real-time quantitative RT-PCR. Br J Cancer. 2006 Jul 17;95(2):218-25	
		MULDER et al., Tumor pyruvate kinase isoenzyme type M2 and immunochemical fecal occult blood test: performance in screening for colorectal cancer. Eur J Gastroenterol Hepatol. 2007 Oct;19(10):878-82	
		REIN et al., Identifying 5-methylcytosine and related modifications in DNA genomes. Nucleic Acids Res. 1998 May 15;26(10):2255-64	
		SHASTRI et al., Comparison of an established simple office-based immunological FOBT with fecal tumor pyruvate kinase type M2 (M2-PK) for colorectal cancer screening: prospective multicenter study. Am J Gastroenterol. 2008 Jun;103(6):1496-504	
		SOMMER et al., Minimal homology requirements for PCR primers. Nucleic Acids Res. 1989 Aug 25;17(16):6749	
		TURGEON et al., Fecal DNA-Based Detection of Colorectal Neoplasia. Curr Colorectal Cancer Rep. 2007 Oct;3(4):171-177	

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		VILLAR-GAREA et al., DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Curr Drug Metab. 2003 Feb;4(1):11-31	

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☐ See attached certification statement.

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

☒ A certification statement is not submitted herewith.

☒ Documents not provided herewith have been previously cited in parent U.S. patent application number 13/147,570 filed on March 12, 2012; 15/010,436, filed on January 29, 2016; 15/634,607 filed on June 27, 2017; and/or 17/936,335 filed on September 28, 2022. In compliance with 37 C.F.R. § 1.98(d), Applicants have not included copies of these documents.

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Name/Print	Mary Ann D. Brow	Registration Number	42363

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(19) [Publication country] Japan Patent Office (JP)
(12) [Kind of official gazette] Published examined patent application (B2)
(11) [Patent number] Japanese Patent No. 4039590 (P4039590)
(24) [Registration date] Heisei 19(2007) November 16 (2007.11.16)
(45) [Publication date] Heisei 20(2008) January 30 (2008.1.30)
(54) [Title of the invention] Stabilization of the hemoglobin sample.
(51) [International Patent Classification]
G01N 33/53 (2006.01)
G01N 33/72 (2006.01)
[FI]
G01N 33/53 K
G01N 33/72 A
[Number of claims] 11
[Total number of pages] 8
(21) [Application number] Japanese Patent Application No. H10-34127
(22) [Filing date] Heisei 10(1998) January 30 (1998.1.30)
(65) [Publication number] JP H 11 - 218533A
(43) [Date of publication of application] Heisei 11(1999) August 10 (1999.8.10)
[Date of request for examination] Heisei 17(2005) January 21 (2005.1.21)
(73) [Patentee]
[Identification Number]000120456
[Name]EIKEN CHEMICAL CO.,LTD.

(72) [Inventor]

[Name]Yukie Yoshizawa

(72) [Inventor]
[Name]** **

(72) [Inventor]
[Name]The 10,000th son of **

[Examiner] TAKENAKA, Yasunori
(56) [Reference]
[Document] JP H 05 - 281227A (JP, A)
(58) [Search field] (Int.Cl., DB name)
G01N 33/53
G01N 33/72

Scope of Claims Close ----

(57) [Claim]
[Claim 1]
To provide a method for stabilizing a hemoglobin sample by coexisting a partial decomposition product of hemoglobin by a proteolytic enzyme in an immunological hemoglobin measurement using an antibody recognizing mammalian hemoglobin.
[Claim 2]
The method for stabilizing a hemoglobin sample according to claim 1, wherein the proteolytic enzyme is trypsin, pepsin or alkalase.
[Claim 3]
The method for stabilizing a hemoglobin sample according to claim 1 or 2, wherein the hemoglobin sample is present in a fecal suspension, and the dispersion medium of the suspension contains a partial decomposition product of hemoglobin by a proteolytic enzyme.
[Claim 4]
A method for stabilizing a hemoglobin sample according to claim 1 or 2, wherein the hemoglobin sample is present in urine and a partial decomposition product of hemoglobin by a proteolytic enzyme is added to the urine.
[Claim 5]
A method of stabilizing a hemoglobin sample according to claims 1 to 4, wherein the hemoglobin sample is human hemoglobin.
[Claim 6]
A dispersion medium for suspending a fecal sample to be tested for the presence of hemoglobin, the dispersion medium comprising:

Dispersion medium containing a partial degradation product of hemoglobin by a leukolytic enzyme
[Claim 7]
The dispersion medium according to claim 6, wherein 10 to 200 μ g of a partial decomposition product of hemoglobin by a proteolytic enzyme is added per fecal 1mg to be suspended.
[Claim 8]
The dispersion medium according to claim 7, wherein the concentration of the partial degradation product of hemoglobin by the proteolytic enzyme in the dispersion medium is 1 μ g/ml or more.
[Claim 9]
The method according to claim 1, wherein the immunological measurement is carried out by immunological aggregation of insoluble carrier particles to which an antibody recognizing human hemoglobin is bound.
[Claim 10]

The measurement method according to claim 9, wherein a mixture of insoluble carrier particles to which a monoclonal antibody r ecognizing human hemoglobin is bound and insoluble carrier particles to which a polyclonal antibody recognizing human hemogl obin is bound is used.

[Claim 11]

The method according to claim 1, wherein the immunoassay is immunochromatography using, as a marker, colored insoluble car rier particles to which a monoclonal antibody that recognizes human hemoglobin is bound.

Detailed Description Close

[Detailed description of the invention]

[0001]

[Industrial field of application]

The present invention relates to a method for stabilizing hemoglobin. Specifically, the present invention relates to a technique for stabilizing hemoglobin in a sample to be detected or hemoglobin as a standard substance used as a positive control. Detection of hemoglobin contained in urine, feces and the like is useful for diagnosis of many diseases. In particular, the detection of hemoglo bin in feces (fecal occult blood) is important information in the diagnosis of digestive diseases including colon cancer. In place of a test paper method based on a chemical color development reaction which has been used for hemoglobin in feces from old time s, in recent years, a detection method by an immunological technique using an antibody specific to human hemoglobin has beco me widespread and has been established as a convenient test method which does not require dietary restriction.

[0002]

[Problems of Related Art]

In order to detect hemoglobin contained in a sample such as feces, it is necessary to transport the sample to a testing facility. A transport container [1] provided with a feces collection mechanism and a feces suspension filtration mechanism is used for trans porting a feces sample. By using this type of container, it is possible to quantitatively collect feces, and it is possible to easily filte r a feces suspension. The container from which the feces has been collected is transported to an inspection facility by means of mailing or the like.

[0003]

During transportation, hemoglobin coexists with bacteria and many other components contained in feces. Further, since it is gen erally difficult to control the temperature, it is inevitable that the product is exposed to a temperature condition which is not pref erable for storage. Thus, there is always the possibility of denaturation and degradation of hemoglobin during transport. Since de naturation and decomposition of hemoglobin during transportation lead to erroneous diagnostic results, it is desirable to minimiz e such denaturation and decomposition.

[0004]

In order to stabilize not only hemoglobin but also protein substances, it is usually effective to add other proteins or sugars. With respect to hemoglobin, it is known that bovine serum albumin (hereinafter abbreviated as BSA), rabbit serum albumin, ovalbumi n or the like as a protein and sucrose or the like as a sugar exhibit a stabilizing effect [2]. The use of animal serum has also bee n reported [3]. However, these general stabilizers have a small hemoglobin stabilizing effect particularly in a fecal suspension, an d sufficient storage performance cannot be expected. Many components such as bacteria and proteolytic enzymes which cause d enaturation and decomposition of hemoglobin are present in feces, and hemoglobin cannot be sufficiently protected only by prot ein and sugar. Further, since animal serum is not a purified pure substance, it has a stabilizing effect.

It is easy to produce a ** difference. In addition, since it cannot be denied that there is a possibility that a component having a denaturation effect on hemoglobin is present in animal serum containing many components, it is hard to say that the stabilizer is a desirable stabilizer. Further, in a report [3] in which stabilization of hemoglobin was attempted with bovine serum, it was neces sary to add a large amount of serum such as 10 to 20% v/v. Stabilization techniques that require large amounts of expensive an imal serum are economically disadvantageous.

[0005]

Techniques for stabilizing hemoglobin in fecal suspensions include the addition of lytic enzymes [4], the use of antimicrobial com

pounds and the like [5] [6], the addition of animal hemoglobin [7] Addition of protease inhibitors [8], control of pH [9], addition of iron protoporphyrin [10], iron-containing proteins such as transferrin [11] [15] and peroxidase [12], and addition of sodium fluoride [14] are known. Alternatively, multi-component combinations [13] have also been tried. It is considered that these inhibit the influence of bacteria or disperse the influence on hemoglobin by a compound structurally similar to hemoglobin, thereby exhibiting a protective effect on hemoglobin.

[0006]

In addition, it is known that ethylenediaminetetraacetic acid (hereinafter abbreviated as EDTA) has a hemoglobin stabilizing effect [16]. However, as a result of additional tests by the present inventors, it was confirmed that EDTA cannot be expected to have a sufficient stabilizing effect on hemoglobin in feces. The Applicant has filed a patent application on the use of a water-soluble metal complex of a transition metal ion which has a higher stabilizing effect than simple EDTA alone [17]. In addition, technique for stabilizing blood protein, which utilizes the fact that antibodies against feces components contribute to the stabilization of blood protein present in feces, was also filed [18].

[0007]

Among these stabilizers, substances structurally similar to hemoglobin, for example, iron porphyrin such as heme and hemin and animal hemoglobin are particularly effective for the antigen stability of hemoglobin in immune reaction. However, heme and hemin are not sufficiently soluble in water, and it is necessary to examine a dissolution accelerator in order to incorporate them into a fecal suspension or the like in an amount sufficient for stabilization. In addition, the addition of heterologous animal hemoglobin is disadvantageous in that cross-reaction cannot be avoided and false positive is likely to occur.

[0008]

[Problem to be solved by the invention]

A first object of the present invention is to provide a novel hemoglobin protective substance. In particular, it is the most important object of the present invention to provide a technique for effectively stabilizing hemoglobin which coexists with feces components having a strong denaturation and decomposition action.

A second object of the present invention is to provide a technique for stabilizing hemoglobin as a sample for analysis. Specifically, an object of the present invention is to provide a technique for stabilizing hemoglobin to be detected in a method for detecting hemoglobin by an immunological method using an antibody that recognizes hemoglobin, which is currently the mainstream.

A third object of the present invention is to provide a dispersion medium for suspending a fecal sample for the purpose of immunological analysis using a novel hemoglobin stabilizer, and a measurement technique using the same.

[0009]

[Means for solving the problem]

The present invention relates to a method for stabilizing hemoglobin by allowing an enzymatic decomposition product of hemoglobin to coexist, and a dispersion medium for suspending a fecal sample to which the stabilization method is applied. In addition, the present invention provides an immunological measurement method using an antibody that recognizes human hemoglobin contained in a biological sample, in which a biological sample stored in the presence of an enzymatic decomposition product of hemoglobin is used as an analysis target.

[0010]

The hemoglobin to be enzymatically degraded may be human hemoglobin or heterologous animal hemoglobin. This is because antigenic globin is degraded by a proteolytic enzyme such as pepsin and loses its antigenicity. The time for pepsin digestion of hemoglobin is suitably about 1 hour at 37 ° C. Globin fragmented below 6kD and antigenic to hemoglobin antibodies

* -- completely ** -- it is divided. When the enzyme reaction is further continued, the globin is further digested to form heme, whereby the solubility is reduced and the auxiliary stabilizing action of the protein (globin fragment) is lost.

[0011]

The present inventors have found that a digestion product by alcalase having an activity in an alkaline condition, which is opposite to pepsin having an activity in an acidic condition, also has substantially the same effect as that of a pepsin digestion product. The hemoglobin decomposition product obtained by this method is commercially available from Asahi Kasei Corporation as HIP (Hem Iron Compound), which is an iron source having excellent absorbability.

[0012]

The method for stabilizing hemoglobin of the present invention is useful for stabilizing hemoglobin as an analyte present in a biological sample. To highly stabilize the antigenicity of hemoglobin to be measured, especially in a method for measuring hemoglobin by an immunological method using an antibody recognizing hemoglobin. Feces and urine are known as biological samples in which hemoglobin is to be detected. Hemoglobin in the stool is indicative of bleeding in the digestive tract, while hemoglobin in th

e urine is indicative of suspected bleeding in the urinary tract.

In particular, hemoglobin in feces is generally identified from hemoglobin derived from a meal by using an immunological metho d having high specificity, and it is highly necessary to stably maintain the antigenicity of hemoglobin.

[0013]

When the stabilization method of the present invention is applied to hemoglobin present in a stool sample, a hemoglobin degrad ation product may be added to a dispersion medium in which the stool is suspended. In the usual detection of hemoglobin in fec es, feces are suspended in an appropriate dispersion medium and filtered as necessary to prepare a sample for immunological a nalysis. It is advantageous to add a decomposition product of hemoglobin to the dispersion medium used at this time. As a resul t of an experiment, the concentration of the hemoglobin degradation product in the dispersion medium is 1 μ g/ml or more.

[0014]

On the other hand, as described above, at present, a simple transport container capable of performing collection, transport, susp ension, and filtration of feces in one container has been put into practical use. This type of container is filled with a dispersion m edium at the time of shipment, and a subject may collect feces by himself / herself and mail the container to an examination faci lity. In general, since the subject is not familiar with such a work, it is necessary to assume a case in which the amount of collect ed feces cannot be strictly controlled. Further, it is desirable to use it in an excessive amount so that there is no problem even if the hemoglobin protective action of the degradation product of hemoglobin is somewhat reduced during transportation. In order to rapidly bring hemoglobin into contact with the degradation product of hemoglobin in the dispersion medium, it is desirable to use an excess amount of hemoglobin. Therefore, for example, when it is assumed that the hemoglobin decomposition product is used for stabilization of human hemoglobin, the hemoglobin decomposition product may be added in a range of 0.1 to 100 μ g, p referably 1 to 30 μ g, with respect to the amount of 1mg feces to be collected, which is assumed at the time of design.

[0015]

In addition to the hemoglobin degradation product which is the protective agent of the present invention, known components ca n be added to the dispersion medium. For example, addition of a buffer giving a pH advantageous for preservation of hemoglobi n, an antibacterial agent for preventing unnecessary proliferation of microorganisms, or many hemoglobin protective component s known so far is also effective. For example, the protective effect of the following components is known:

Examples of the inactive protein having a hemoglobin stabilizing action include serum albumin derived from human, bovine, rabb it, horse, sheep, goat, or the like, albumin derived from egg white, and the like. Amino acids such as lysine and histidine are also found to have a protective effect on hemoglobin. Known antimicrobial agents include lytic enzymes [4], azides, ethyl benzoate, p enicillin, fungizone [5], streptomycin, or cephamycin and other non-penicillin antibiotics [6]. It is also known that protease inhibi tors such as trypsin inhibitor and α 2 macroglobulin stabilize hemoglobin [8]. Sodium fluorides [14] or water-soluble metallic co mplexes of transition metal ions such as the FeIIIEDTA complex [

17] also reports a stabilizing effect on hemoglobin. Further, salts or the like for adjusting ionic strength may be added.

[0016]

An example of a specific composition of the solution for stabilizing hemoglobin according to the present invention is shown belo w.

Degradation products of hemoglobin: 100 μ g/ml

HEPES buffer (pH7. 4): from 10 to 500mM

BSA:0. 1-5%

NaN3:0.1-1 %

[0017]

The pH of the dispersion medium is set within a range in which hemoglobin can be stably retained. Under extreme acidic or alkali ne conditions, the stability of hemoglobin may be impaired, and the digestive enzyme used for the degradation may recover its a ctivity, so that a pH in the neutral range is desirable. Specifically, the pH may be about 5 to 10, preferably about 6 to 8. A suitabl e buffer is utilized to maintain the pH. For example, hydroxylethyl piperazine-2-ethanesulfonic acid (abbreviated as N-2 Hydroxy ethylpiperazine - N' - 2 ethanesulfonic acid ,HEPES); A Good buffer such as piperazine-bis(2-ethanesulfonic acid) (Piperazine - N, N' - bis (abbreviated as 2 - ethanesulfonic acid),PIPES)) can be used in combination with A particularly preferred buffering ag ent is one which provides a pH (6 to 8) at which the structure of hemoglobin is most stabilized and which is also used as a reacti on buffer when hemoglobin is detected by an immune reaction. In addition, a phosphate buffer solution, a Tris buffer solution, a glycine buffer solution and the like can also be used.

[0018]

The method for stabilizing hemoglobin of the present invention can be used for stabilizing hemoglobin in a fecal sample for the p

urpose of detecting stools fecal occult blood. In particular, the present invention is useful for maintaining the antigenicity of hem oglobin as an immunological analyte for which protection of the antigenic structure is required.

[0019]

The present invention provides an immunological method for measuring hemoglobin using the hemoglobin stabilization techniqu e. Examples of the immunological measurement method include a latex agglutination reaction method, a gold colloid agglutinatio n reaction method, an immunochromatography method, and an ELISA method. In any of the measurement methods, the presen ce of the degradation product of hemoglobin in the hemoglobin-containing sample protects the antigen activity during storage su ppresses a decrease in the measurement value.

[0020]

It is also possible to construct an agglutination reaction system only with a monoclonal antibody. In order to carry out an aggluti nation reaction only with a monoclonal antibody, it is advantageous to combine a plurality of monoclonal antibodies that recogniz e different epitopes on hemoglobin. Hemoglobin has a tetrameric structure in which two α chains and two β chains are associate d with each other, and in principle, even one kind of monoclonal antibody aggregates. However, since hemoglobin is in an equilib rium state of a dimer structure and a tetramer structure of α β in a solution, a single monoclonal antibody cannot react with all o f them. In addition, since a single monoclonal antibody cannot react when a target epitope is denatured, it is stochastically adva ntageous to use a plurality of kinds of monoclonal antibodies in combination.

[0021]

[Action and effect]

The degradation product of hemoglobin in the present invention has an action of effectively suppressing a decrease in the measu red value of hemoglobin during the storage period. In particular, it exhibits a sufficient protective effect against hemoglobin in a f ecal suspension which contains a large amount of denaturation / decomposition components and is difficult to control the storage conditions.

It is not known by what mechanism the degradation products of hemoglobin protect hemoglobin.

[0022]

[Effect of the Invention]

According to the technique for stabilizing hemoglobin of the present invention, a protective action can be obtained even in the pr esence of a feces components having a strong denaturation / decomposition action on hemoglobin. Therefore, the part of feces o ccult blood

This method is useful for stabilizing hemoglobin contained in a sample to be precipitated. In particular, the present invention con tributes to the maintenance of antigenicity of hemoglobin as an immunological analysis target for which protection of an antigen structure is required. According to the present invention, hemoglobin in a stool sample is effectively stabilized, and prevention of false negative results due to denaturation and decomposition of hemoglobin can be expected. The enzymatic decomposition prod uct of hemoglobin required in the present invention is economically advantageous because the raw material and the enzyme use d for decomposition are inexpensive. Further, since the decomposition product of hemoglobin exhibits a high stabilizing effect ev en in a small amount, it can also be said to be an economically advantageous stabilizing technique.

The invention the present invention will be described in more detail with reference to examples.

[0023]

[Examples]

1. Production of enzymatic degradation products of animal hemoglobin

Sodium citrate was added to the swine bloods collected from the slaughtered swine to a concentration of 0.3%, and swine hemo cytes were obtained by centrifugation (6000rpm, 20 minutes). The porcine blood cells were hemolyzed by repeated freeze-thawi ng. About 8ml of conc. hydrochloric acid was added to the 1000ml of the hemolyzed blood to adjust pH1, and 50g of pepsin pow der (1200U/mg, Sigma) was added thereto, followed by gentle stirring at 30 ° C. for 1 hour, and then 1mol of NaOH was added t o neutralize the hemolyzed blood to stop the enzyme reaction. Subsequently, a fraction having a molecular weight of 5000 to 60 00 was collected using an ultrafiltration membrane (manufactured by SIP1013 Asahi Kasei Corporation) and freeze-dried.

[0024]

2. Hemoglobin Stabilization by Hemoglobin Degradation Products

The enzymatic decomposition product of bovine hemoglobin obtained in Example 1 and HIP (manufactured by Asahi Kasei Corpo ration) were each added to fecal suspensions (HEPES buffer (pH7. 4) 100mM,BSA 1%,NaN3 0.3 %) at a concentration of 100 μ g/ml, and the stabilities of the fecal suspensions alone and hemoglobin were compared.

Two above-mentioned type of buffer solution [2 ml of] was dispensed to each stool receptacle of OC-*****' Nutrition research

'(made by EIKEN CHEMICAL), human feces were extracted in the container according to the operation manual, and the amount of hemoglobin 24 hours immediately after extraction and after 37 degree C was measured by the facilities occult blood measuring apparatus OC-sensor II (EIKEN CHEMICAL sale). The results are shown in Table 1.

[0025]

[Table 1]

糞便中ヘモグロビンの安定性

単位:ng/ml

	懸濁液のみ		牛Hb酵素分解物添加		HIP添加	
	0hr	24hr	0hr	24hr	0hr	24hr
便1	742	31	738	293	739	273
便2	747	76	745	307	755	322
便3	701	154	636	469	668	454
便4	766	133	729	433	741	420
便5	717	100	679	244	683	221
便6	705	111	695	415	690	397
便7	796	251	801	510	815	506
平均	739	122	718	382	727	370

[0026]

While the hemoglobin in the suspension alone decreased to an average of 16.5% after 24 hours at 37 ° C., the hemoglobin in the buffer obtained by adding the enzymatic decomposition product of bovine hemoglobin to the suspension remained in an average of 53%, and the hemoglobin in the buffer obtained by adding HIP remained in an average of 50%. Both of them were confirmed to have an excellent stabilizing effect on the antigenicity of hemoglobin.

[0027]

3. Confirmation of cross-reactivity

Hemoglobin concentration 5000 μ g/ml obtained by adding bovine hemoglobin and porcine hemoglobin to the fecal suspension

Each stock solution was diluted by two fold with the fecal suspension to prepare a dilution series. Similarly, enzyme decomposition products (pepsin treatment) of bovine hemoglobin and porcine hemoglobin corresponding to a hemoglobin concentration of 5000 μ g/ml were prepared, and a dilution series was prepared for each. Their reactivity with human hemoglobin antibody was measured with OC Sensor II. The results are shown in Table 2.

[0028]

[Table 2]

ヒト・ヘモグロビンとの交差反応

	牛-ヘモグロビン		豚-ヘモグロビン	
Hb含有量	未処理	酵素分解物	未処理	酵素分解物
(μg/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
20	17	0	67	3
39	35	0	128	2
78	40	0	176	7
156	65	0	244	2
313	106	0	294	4
625	149	2	291	5
1250	173	8	235	11
2500	163	19	157	13
5000	122	34	85	21

[0029]

Due to the prozone phenomenon, the linearity at a measured value of 100ng/ml or more was lost, but about 1 / 1000 of the added amount of bovine hemoglobin showed cross-reactivity with human hemoglobin antibodies, and 1 / 300 of the added amount of porcine hemoglobin showed cross-reactivity. On the other hand, the cross-reactivity of the pepsin-digested hemoglobin degradation product is reduced to 1 / 100,000 or less. It was confirmed that the addition concentration 10-200μg/ml of the hemoglobin decomposition product showing the hemoglobin stabilization effect did not affect the measurement value.

[0030]

Cited document

[1] JP H 5 - 17652Y
[2] JP S 63 - 243756A
[3] JP H 4 - 145366A
[4] JP H 5 - 69466B
[5] JP S 63 - 271160A
[6] JP H 7 - 72154A
[7] JP H 2 - 296149A
[8] JP H 3 - 279859A
[9] JP H 5 - 281226A
[10] JP H 5 - 281227A
[11] JP H 8 - 29429A
[12] JP H 8 - 29430A
[13] JP H 6 - 281654A
[14] JP H 7 - 191026A
[15] JP H 8 - 262020A
[16] JP H 2 - 221859A
[17] JP H 7 - 229902A
[18] Japanese Patent Application No. H7-302051

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
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PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
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				Filing Date	03/07/2023
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	1	of	2	Attorney Docket Number	EXCTD-35239.307

U.S. PATENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4859610	1989-08-22	MAGGIO	
		5137806	1992-08-11	LEMAISTRE et al.	
		5196167	1993-03-23	GUADAGNO et al.	
		5527676	1996-06-18	VOGELSTEIN et al.	
		5741650	1998-04-21	LAPIDUS et al.	
		5952178	1999-09-14	LAPIDUS et al.	
		11634781	2023-04-25	LOUWAGIE	

U.S. PUBLISHED PATENT APPLICATIONS					
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FOREIGN PATENT DOCUMENTS						
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		EP 1366715	2003-12-03	SENTINEL CH. S.r.L		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		

NONPATENT LITERATURE DOCUMENTS			
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				Art Unit	
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Sheet	2	of	2	Attorney Docket Number	EXCTD-35239.307

NONPATENT LITERATURE DOCUMENTS			
		AHLQUIST et al., Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia. Ann Intern Med. 2008 Oct 7;149(7):441-W81	
		HOEPFFNER et al., Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study. Aliment Pharmacol Ther. 2006 Jan 1;23(1):145-54	
		LEUNG et al., Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of Patients with Colorectal Cancer or Polyps. Am J Gastroenterol 2007;102:1070-6	
		OLSON et al., DNA Stabilization is Critical for Maximizing Performance of Fecal DNA-Based Colorectal Cancer Tests. Diagn Mol Pathol 2005;14:183-191	
		Request for Ex Parte Reexamination of U.S. Patent 11,634,781, filed May 22, 2023, 286 pages	
		Declaration of Mr. Anthony P. Shuber, MS, Reexam Application 90/015,237, filed May 22, 2023, 63 pages	

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(71) Applicant (*for all designated States except US*): **LOOTS LIMITED**; PO BOX 293, GRANITE HOUSE, LA GRANDE RUE, ST MARTIN GUERNSEY GY1 3RS (GB).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **O'NEILL, IAN** [GB/GB]; 5 RED HILL CLOSE, GREAT SHELFORD, CAMBRIDGE Cambridgeshire CB2 5JP (GB).

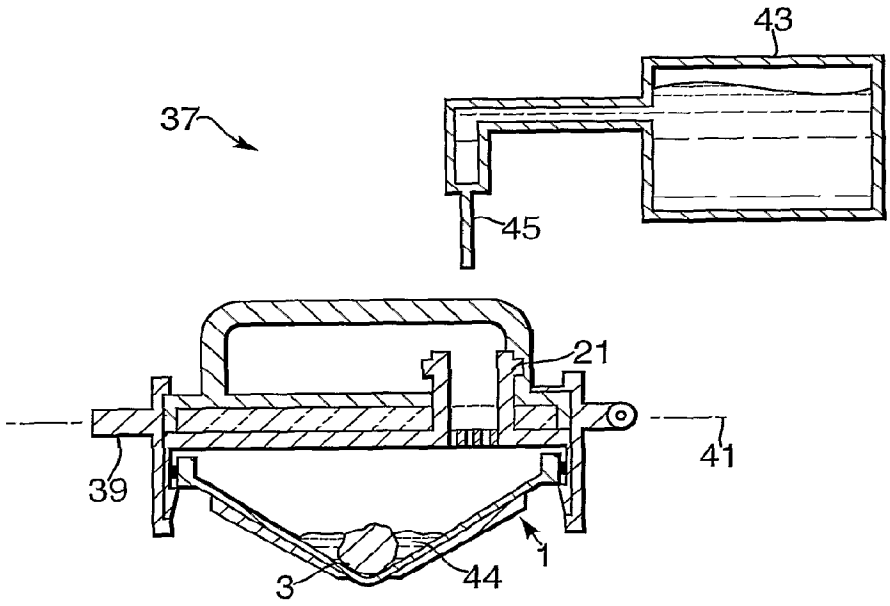
(74) Agents: **GILL, Stephen** et al.; Mewburn Ellis LLP, York House, 23 Kingsway, London Greater London WC2B 6HP (GB).

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[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR REMOVING CELLULAR MATERIAL FROM FAECAL STOOLS AND CONTAINER FOR COLLECTING FAECAL STOOLS



(57) Abstract: A method of removing cellular material from the surface of a faecal stool comprises the steps of: a) supporting the stool in a container having a channel along which the stool extends; b) cooling the stool to a temperature below its gel greezing point; c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool. Further disclosed are an apparatus (4) for removing cellular material from. the surface of a faecal stool and a container for collecting faecal stools (3b) .

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METHOD AND APPARATUS FOR REMOVING CELLULAR MATERIAL FROM FAECAL STOOLS AND CONTAINER FOR COLLECTING FAECAL STOOLS

Field of the Invention

The present invention relates to a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools.

Background

Faecal stools are the residue of digestive products in the gastrointestinal tract, and contain not only bacteria and undigested food residues, but also residual products from the body's metabolism and structure such as exfoliated epithelial cells from the lining of the gastrointestinal tract.

Exfoliated epithelial cells consist of cellular components, such as DNA, RNA and proteins, which potentially provide a multiplicity of approaches to identifying and monitoring unhealthy conditions of the epithelium as exemplified by colorectal cancer, polyps and ulcerative colitis. The facile recovery of such cells makes possible the application of a wide variety of test procedures for qualitative and/or quantitative analysis of, for example, DNA alterations, gene expression and enzyme activity. A. Loktionov *et al.*, in *Clinical Cancer Research*, Vol. 4, 337-342, (1998), describe a non-invasive screening test for early detection of colorectal cancer which is based on a quantitative comparison of the DNA concentrations derived from exfoliated epithelial cells isolated from the surface of stools from healthy and tumour-bearing persons. In this isolation procedure, cells on the surface of the stool were predominantly clustered and had varying degrees of intactness and damage. In the present specification by "cells" and "cellular material" we mean all such material irrespective of degree of clustering or intactness.

In our earlier patent application, WO 97/09600, we describe a method of isolating cells (especially exfoliated epithelial cells) from the surface of faecal stools, comprising cooling a stool to a temperature (preferably of -80°C to 15°C, preferably -10°C to 15°C, preferably -10°C to 10°C, more preferably 0°C to 5°C) below its gel freezing point and removing the cells from the cooled stool by agitating the stool in an aqueous solution (which may be pre-cooled to a temperature below the gel freezing point, and is preferably ice-cold). The cooled condition creates a much more physically robust hard gel state that helps to prevent the stool from breaking up or rapidly eroding so that non-surface material is largely prevented from entering the aqueous solution.

Stool surface layers provide a particularly rich source of exfoliated cells suitable for isolation. Typically between one-sixth and one third of colonic epithelial cells are exfoliated per day (D. Sidransky *et al.* (1992), *Science* 256, 102-105 and W. C. MacDonald, J. S. Trier, N. B. Everett, *Gastroenterology* (1964) 46, 405). It is believed that some of these exfoliated cells, together with mucus, may be envisaged as forming a "sheath" around a stool, containing a low proportion of bacterial cells and other faecal matter. It is further believed that the viability of the cells in the surface layers (i.e. the layer of faecal material within 1.0 mm, and preferably 0.25 mm, of the stool surface) of the stool is facilitated by i) a tendency of the cells to agglomerate into plaques (which helps to prevent apoptosis), ii) the availability of oxygen at the surface of the stool and iii) the unique ability of colonic epithelial cells to utilise as fuel the butyrate formed locally in high concentrations by microfloral fermentation (J. H. Cummings (1981) *Gut* 22, 763-799 and W. E. W. Roediger (1980) *Gut* 21, 793-798). Oxidation of butyrate is believed to provide fuel for the cells in the

absence of blood-borne nourishment following exfoliation. It has been found that isolation of cells from stool surface layers provides significant advantages in yield, purity and ease of recovery over isolation of cells from homogenised stools.

Summary of the Invention

Although the method described in WO 97/09600 is effective for isolating cells from the surfaces of faecal stools, in order to make the method more suitable for high throughput screening of e.g. hundreds or thousands of samples per day, problems remain of washing the stools in a standard and reproducible way that maximises the amount of surface cells extracted from the stools but minimises the risk of breaking up the stools.

Consequently, in a first aspect, the present invention provides a method of removing cellular material from the surface of a faecal stool comprising the steps of:

a) supporting the stool in a container having a channel along which the stool extends;

b) cooling the stool to a temperature below its gel freezing point;

c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and

d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool. A minority of stools are very soft when excreted, such that on cooling they do not reach the gel freezing point, but do become sufficiently viscous to be washed with minimal entry into the wash liquid of sub-surface material. Thus, for that minority of stools, by "gel freezing point" we mean the temperature at which the stools become sufficiently viscous to be washed with negligible entry of sub-surface material into the wash liquid.

The method promotes the reproducible and gentle washing of the stool, which facilitates the quantitative comparison of cellular material between individuals.

Human faecal stools are typically elongate and approximately cylindrical in shape. So the channel significantly reduces the likelihood that sections of the stool will break off when the stool is being washed because the channel walls substantially prevent side-to-side rolling motion of the stool and, for a wide range of rocking angles and speeds, friction largely prevents the stool from sliding along the channel. Preferably the container is moved to cause a wash frequency of from 5 to 40 back and forth cycles per minute, and more preferably from 15 to 25 cycles per minute. The stool may be pre-soaked (for e.g. 3 minutes) in the wash liquid prior to step d), in which case preferably the container is moved for a total time of from 10 seconds to 1 minute. Without a pre-soak, preferably the container is moved for a total time of from 30 seconds to 5 minutes. With or without pre-soaking it is advantageous to limit the exposure of the stool to the wash liquid so as to avoid localised depletion of wash reagents or over-enrichment of released cellular material. The channel may have a V or U-shaped transverse section.

An advantage of the container motion, is that the wash liquid is channelled back and forth along the length of the stool so that, compared with e.g. circular agitation by an orbital shaker of the wash liquid relative to the stool (which we have found in any event tends to break up the stool), less liquid is required to wash the cells from the surface of the stool. Clearly the less liquid that is used to wash the stool, the higher the resulting concentration of cells in the liquid, which can in turn improve the accuracy of screening tests which may be subsequently performed on the liquid. Also smaller volumes of liquid

reduce cost and disposal problems. Preferably from 30 to 150 ml of liquid is used in steps c) and d). This is generally sufficient to wash a typical 100 g stool while minimising losses due to residual wetting of the container and stool. Most preferably a fixed ratio of liquid to stool is used so that all stools experience a similar degree of wetting. This ratio may be in the range 0.5 to 1.0 ml of liquid per gram of stool.

In one embodiment, step d) comprises rocking the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity. In another embodiment, step d) comprises displacing the container back and forth in the direction in which the channel extends.

Preferably the container is of fixed shape, e.g. by being formed of rigid material.

In a second aspect, the present invention provides an apparatus for removing cellular material from the surface of a faecal stool, comprising:

mounting means for mounting a container having a channel along which, in use, the stool extends; and

motion imparting means for moving the container so that, when wash liquid is introduced into the container, the liquid runs back and forth along the channel thereby washing cellular material from the surface of the stool.

In one embodiment, the motion imparting means rocks the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity. In another embodiment, the motion imparting means displaces the container back and forth in the direction in which the channel extends.

The apparatus may further comprise control means to control e.g. the rocking angle, rocking or displacement frequency and/or total time of movement of the container, preferred ranges for some of these parameters being

provided above in respect of the first aspect of the invention. The control means may further control a sequence of stationary and mobile periods for the container, so that e.g. stationary exposure of the stool to the wash liquid allows the liquid to penetrate the stool and loosen its surface layers prior to active washing when the container is moved.

With such an apparatus, stool washing can be performed in a repeatable, standardised manner, and with a relatively high stool sample throughput. These are significant advantages if the wash liquid is to provide the material for a screening test based on e.g. a quantitative comparison of amounts of epithelial cell components.

The apparatus may further comprise cooling means for cooling the stool to a temperature (preferably of -80°C to 15°C, preferably -10°C to 15°C, preferably -10°C to 10°C, more preferably 0°C to 5°C) below its gel freezing point.

In one embodiment the apparatus further comprises a filter means such as a sieve, so that, when wash liquid is removed from the container, the cells are retained in the liquid but food residues from the stool are filtered from the liquid.

The apparatus may further comprise a metering device for introducing a metered dose (e.g. 50 to 150 ml, or an amount which is related to the stool weight) of wash liquid into the container, and/or a wash liquid pre-cooler for cooling the wash liquid to a temperature below the gel point of the stool before the wash liquid is introduced into the container. This temperature is preferably about 0°C.

The apparatus may further comprise at least one and preferably a plurality of detachable sample receptacles into which wash liquid is transferable from the container, so that e.g. when stool washing is completed the wash liquid may be poured from the container into the

receptacles. These can then be detached from the apparatus and transferred to an automated liquid-handling system for high throughput processing of the liquid samples contained therein.

A third aspect of the invention provides a fixed shape container for collecting faecal stools which has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel. The mouth can be of any suitable shape, e.g. rectangular or circular. The length of the channel is preferably in the range from 10 to 25cm, which corresponds to the size of a typical stool. The container may be used in the first or second aspect of the invention.

As faecal stool is generally regarded as disgusting material that people are reluctant to handle, even indirectly, the fixed shape container has several advantages as compared with, e.g. a flexible plastic specimen bag for collecting stool. For example, the mouth of the container cannot collapse as a stool is being deposited in the container, thereby making it easier for a user to excrete into the container. Also the container can be handled without, apart from the weight of the stool, any sensation that a stool is contained therein.

Furthermore the funnelling effect of the narrowing container walls guides a formed stool (which on defecation can be quite malleable) to a position in which it extends along the channel. This reduces or eliminates the need for the user to directly or indirectly manipulate the stool into that position prior to stool washing. Soft stools, and even diarrhoea, are also encouraged to align along the channel by the narrowing of the container walls.

A further advantage of the container is that it can serve both as the receptacle into which the stool is

excreted and as the container in which the stool is washed. So the stool does not need to be transferred to a different washing container, which avoids damage to the stool surface caused by such a transfer. Such damage would interfere with the accuracy of e.g. screening tests which may be performed on the stool.

If the stool is excreted into the container at home and then transported to e.g. a clinic for washing, the container has the further advantage of holding the stool reasonably securely in the channel so that transportation damage to the stool surface is reduced or avoided.

To avoid user embarrassment during transportation at least a majority of the container may be opaque (i.e. a stool inside the container is not visible through the opaque portions of the container, even if the material of the container transmits light to some degree), or an opaque transport casing may be provided for transporting the container. However, desirably at least a portion of the container permits viewing to the inside of the container so that e.g. after delivery of the container for stool processing a health professional can check the stool for gross abnormalities such as blood before the stool is processed and without opening the container.

The container may further have installation means (which may be detachable from the container) for removably installing the container on a toilet (e.g. below the toilet seat) with the receiving mouth opening upwardly and the collection channel extending horizontally beneath the mouth. This allows the user to produce the stool in the usual manner, which promotes user acceptability.

Typically, for hygiene reasons, at least the stool-contacting parts of the container are disposable. However, in one embodiment the internal walls of the container serve as a former for a bag which is reversibly fixable inside the container, whereby the bag assumes the shape of the

mouth and channel. In this embodiment the bag is typically disposed after use, but the container can be reused.

Preferably the container is made of plastics material such as polypropylene. Desirably the container has smooth internal surfaces which reduce damage to the surface of the stool as the stool passes from the mouth to the channel.

In one embodiment the container has cooling means, such as an outer jacket or bath, for cooling a stool deposited in the container. This means that the stool can be cooled immediately it is produced, which helps to preserve the surface layer cells and makes the stool robust for physical transport. The cooling means may be of material that can be pre-cooled in a domestic refrigerator or freezer. This is particularly convenient when the stool is produced at home, allowing the donor to defecate at a convenient time and in a minimally stressful setting and thereby promoting user acceptability. The cooling means may enable the stool to be cooled to a temperature below its gel freezing point.

The cooling means may be detachable from the container so that the cooling means can be pre-cooled before fitting to the container. Also, if the container is disposable, the cooling means can still be reused.

Anaerobic bacteria in stools produce damaging radicals when exposed to atmospheric oxygen (C. F. Babbs (1990) *Free Radical Biology and Medicine*, 8, 191-200). Such oxygen also oxidises the mucus on the surface of stools and encourages the growth of aerobic bacteria. So preferably the container is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein. The seal may be achieved by means of a lid or cover (e.g. having a snap-fit or screw fitting) for the mouth. A sealed container rapidly becomes anaerobic due to the high concentration of anaerobic bacteria in the stool, and this apparently largely prevents the growth of aerobic

bacteria on the stool surface, helping to preserve the stool. We have found that sealed containers allow stools to be stored at 0°C for a number of weeks apparently without significant degradation. To promote user acceptability, the seal should also eliminate any odour from the stool escaping from the container, particularly if the stool is produced at home and then transported before washing.

The container may also have a liquid port which allows wash liquid to be removed from the container while maintaining the stool in the container. For example the diameter of the port may be sized to prevent a typical-sized stool from exiting via the port, or a foraminous screen over the port may serve the same function. The port may include closure means such as a valve or a cap which allows the liquid to be poured or sucked out of the container but at other times maintains an airtight seal. The same or another port may be used to introduce wash liquid into the container.

A further aspect of the invention provides a kit of parts comprising the container of the previous aspect with the installation means and/or the cooling means and/or the transport casing.

Another aspect of the invention provides a method of screening for unhealthy condition(s) of the epithelium (such as colorectal cancer, polyps and ulcerative colitis) in a plurality of subjects (which are typically human subjects, but may be other animal species such as dogs and cats), the method comprising the steps of:

a) providing to the subjects respective containers (e.g. according to the third aspect of the invention) for defecation of a stool sample thereinto at the convenience of the subject (which is typically when the subject is "at home" or in a domestic setting);

b) collecting the filled containers from the subjects at a central location (which may be a doctor's surgery or a hospital out-patients clinic);

c) removing a specimen of cellular material from the surface of each stool sample (e.g. according to the first aspect of the invention); and

d) testing each specimen for unhealthy condition(s) of the epithelium (e.g. as described by A. Loktionov *et al.*, referenced above).

Brief Description of the Drawings

The present invention is now described in respect of a specific embodiment and with reference to the accompanying drawings in which:

Figs. 1a and b show schematically and respectively a cross section through and a plan view of a receptacle for collecting a faecal stool, and Fig. 1c shows a plan view of an alternative form of the receptacle;

Fig. 2 shows the same cross section as Fig. 1a except that a lid is fixed to the receptacle;

Fig. 3a shows a schematic cross section of the receptacle of Fig. 1a fixed inside a transport unit, and Fig. 3b shows the receptacle of Fig. 1c fixed in another version of the transport unit;

Fig. 4 shows schematically a stool washing apparatus with the receptacle of Fig. 1a positioned in a sample rack; and

Fig. 5 shows the apparatus of Fig. 4 with the sample rack pivoted to pour wash liquid out of the receptacle.

Detailed Description

Figs. 1a and b show respectively a cross section through and a plan view of a transparent plastic receptacle 1 having a V-shaped cross-section for collecting a faecal stool 3 produced by a user. The receptacle has a mouth 5 and a straight stool collection channel 7 (which extends perpendicularly to the plane of the paper in Fig. 1a). The

receptacle is removably installed inside a toilet bowl 9 by a support web 11 which is attached over the rim of the toilet and has a central hole 12 to receive the receptacle so that channel 7 is horizontal and mouth 5 is directly above channel 7. The smoothly sloping walls 14 of the receptacle guide the stool to the bottom of channel 7 and encourage the deposited stool to align with the longitudinal direction of the channel.

Fig. 1c shows an alternative form of the receptacle which has a U-shaped channel cross-section, and which is capable of receiving larger specimens.

Once the stool is collected in the receptacle, a lid 13 (see Fig. 2) is used to close mouth 5 and is releasably fixed to the receptacle by a snap-fit mechanism 16. A sealing ring 15 seals the receptacle from the external atmosphere.

Lid 13 includes a carrying handle 17, thermally insulated upper wall 19 and wash liquid entry/exit port 21. The port is sealed by closure cap 23 and has a foraminous screen 25 at its inner end which prevents the stool from entering the port when (as described below) wash liquid is decanted from the receptacle, and a coarse sieve 25a to retain particles of e.g. greater than 2 mm in diameter.

The receptacle is lifted by handle 17 from web 11 and fixed inside a transport unit 27 by a snap-fit mechanism 29 (see Fig. 3a). The transport unit has thermally insulated walls 31 and contains a cooling block 33 of pre-cooled ice or chemical cooling medium contained under a shaped divider 35. The cooling block is brought into close proximity with the outer wall of receptacle 1. Ring 15a maintains the position of channel relative to the cooling block. The cooling block cools stool 3 to a temperature preferably in the range from 0 to 5°C, which is below the gel freezing point of the stool.

Fig. 3b shows the receptacle of Fig. 1c fixed in another version of the transport unit. In this version divider 35 forms an air space around the receptacle. The air space and divider 35 prevent the cooling block cooling the stool so rapidly as may damage the outer surface layers of the stool.

The stool is then transported in the transport unit to e.g. a clinic where stool washing is performed. The walls of the transport unit and lid are opaque so that the stools are not visible inside the unit, and lid 13 prevents odours from escaping the unit. Channel 7 also reduces movement of the stool during transportation which prevents significant damage to the surface of the stool.

At the clinic, the receptacle 1 and lid 13 are removed from the transport unit. As the receptacle is transparent visual inspection of the stool is possible without removing the lid. At this stage the stool sample may be rejected if, for example, it is too small or runny. also any gross evidence of disease, such as blood, may be noted. However, if the stool is acceptable, the receptacle and lid are positioned into the sample rack 39 (i.e. receiving means) of a stool-washing apparatus 37 shown in Fig. 4. Collection channel 7 is aligned perpendicular to axis 41 about which rack 39 is pivotable and which at this stage is horizontal.

Cooling means (not shown), such as a cold air jet, refrigerator unit or coolant bath, keeps or brings the stool to a temperature in the range 0 to 5°C so that it is below its gel freezing point.

Apparatus 37 has a reservoir 43 of chilled wash liquid, such as a buffered aqueous solution containing N-acetylcysteine, bovine serum albumin and sodium butyrate which has been found (see WO 97/09600) to improve recovery of exfoliated cells. When the stool is sufficiently cool, closure cap 23 is removed and a metered dose of wash liquid

44 is injected into the receptacle via an injector 45 and port 21. The liquid may alternatively be injected through an air-tight septum at port 21. Afterwards injector 45 is swung away from the sample rack.

Rocking means (not shown), which is e.g. an electric motor and associated drive, then rocks the sample rack and receptacle backwards and forwards about axis 41. A control unit (also not shown) which controls the rocking means defines a wash programme consisting of rocking angle, rocking frequency and total exposure and rocking time experienced by the stool. As receptacle 1 moves, gravity causes wash liquid 44 in the receptacle to run back and forth along channel 7 so that intimate contact with the stool is achieved and surface cells from the stool are washed into the liquid. To a significant extent friction prevents the stool from moving during the rocking procedure.

When the wash programme ends, the sample rack is pivoted about pivot 47 to bring axis 41 into a vertical position (see Fig. 5). Almost all of wash liquid 44 is then decanted out of receptacle 1 via port 21, foraminous screen 25 substantially preventing larger food residues or other broken off pieces from the stool exiting with the liquid. Coarse sieve 25a removes smaller pieces from the liquid. Finer downstream sieves (not shown) further limit the maximum particle size in the liquid. The liquid is then be divided into standard volumes and received into a plurality of removable (e.g. clip-on) sample receptacles (not shown). The sample receptacles may be a strip of tubes each of e.g. 0.1-0.5 ml volume as can be removed and clipped into a standard microplate format used for clinical analyses. Alternatively the sample receptacles may be individual tubes each of e.g. 1-2 ml volume. The sample rack is then returned to its horizontal position and receptacle 1 is removed and safely disposed of.

In this way, the apparatus converts a typical total 100-500 g mass of stool and wash liquid into aliquots of cell-suspension of typically only 50-1000 mg (i.e. a thousand-fold reduction in scale). The aliquots can be made representative by preventing the cells from settling out during the decanting process the aliquots. One method of preventing settling out is to provide mixing channels (not shown) through which the liquid flows on its way to the sample receptacles. Alternatively a stirrer (also not shown) can be provided to stir the wash liquid. Fine sieves (e.g. of water wettable material such as nylon, and/or having a mesh size in the range 50-400 μm, preferably 100-250 μm, more preferably 125-200 μm) may be provided over the entrances to the receptacles to further filter the liquid. The excess liquid flows to waste.

The samples may be used for e.g. DNA assays as described in WO 97/09600 and A. Loktionov *et al.*, *Clinical Cancer Research*, Vol. 4, 337-342, (1998).

The entire procedure, from when the receptacle is positioned in the sample rack to when the wash liquid exits the receptacle, can be automated, with standard wash programme settings being used. Also no manual contact with or manipulation of the stool required, which promotes good hygiene. So the present invention allows a large number of stool samples to be processed in a safe and reproducible manner.

In an experimental study, stools were collected into re-sealable bags and stored at 0°C from no more than a few minutes after excretion. The bags were transparent, which allowed visual inspection of the stools during testing. For stool washing, a wash solution was poured into each bag and the bag was located inside a rigid rocking channel of a stool washer apparatus, the channel being 40-45mm wide and perforated so as to permit intimate cooling by surrounding

ice-water held in an insulated container equipped with a submersible pump. Rocking caused a gravity-imposed back and forth circulation of the wash solution along the channel and also promoted cooling of the stool by the surrounding ice-water. A frequency of 3 seconds per rocking cycle was chosen.

The wash solution was as previously described by A. Loktionov *et al.* (referenced above), but the stool wash was passed through a 250 µm instead of a 125 µm sieve. Cell clean-up was performed robotically on a TECAN™ Genesis RSP 150 and then DNA was released by mechanical cell disruption in the presence of 0.9%SDS as lysing agent. Following dilution to prevent quenching, fluorimetric DNA assay was performed with a TECAN™ Spectrafluor Plus using PicoGreen (Molecular Probes™) to yield the stool DNA index (SDNAI, defined as nanograms of DNA per ml of stool wash).

The effects of pre-soaking, rocking angle, mechanical and bacterial damage, stool age, and wash volume were studied, and the results are given below.

Effect of pre-soaking on SDNAI

The following SDNAI data from the stool washer (using a +/-45deg rocking angle) demonstrate the advantage of a period of pre-soaking. Presoaking appears to promote a more or less equilibrated suspension state of cellular material in the wash solution, which thereby helps to prevent the chance dynamic release of cellular material.

Rocking time	SDNAI (ng/ml) with no pre-soak			SDNAI (ng/ml) with 3 min pre-soak		
	stool 1	stool 2	stool 3	stool 4	stool 5	stool 6
30-40 sec	34	12	29	115	206	217
1 min	118	43	21	101	221	294
3 min	100	80	75	125	216	338

Effect of Rocking Angle

If too small a rocking angle was used or the channel became obstructed by the stool, the wash liquid tended to form non-flowing pools that failed to contact the stool surface so that unacceptable variations in washing condition were experienced. However, under favourable conditions, reproducible and relatively constant results could be obtained over a range of rocking angles. For example in three different experiments the same stool was given a 3 minute pre-soak followed by a 1 minute of wash at different rocking angles in a visibly free flow of liquid in a channel 40-45 mm wide and using a wash liquid to stool ratio of 0.7 ml per gram. The rocking angles for the three experiments were +/-30, 45 and 60° from the horizontal and the respective SDNAI measurements were 263, 254 and 260 ng/ml.

Effects of Damage to Stool Surface Layer and of Stool Age

Sometimes stored stools were found to be accidentally squashed, or stored in improperly sealed containers as evidenced by smalls amount of white bacterial over-growth on the stools. The following data (using different stools all washed for 1 minute after a 3 min pre-soak at a wash solution/stool weight ratio of 0.7) demonstrate the importance of protecting the stool surface layer during storage. However, they also demonstrate that under favourable conditions storage durations of up to several weeks have no particular effect on measured SDNAI values. The squashed stools had SDNAI values significantly above the 700 ng/ml value which is considered to be the upper limit for healthy persons.

Stool age (days) donor A	1	4	7	11[s]	15	19	81[s]
SDNAI	203	192	90	1,400	218	585	987

Stool age (days) donor B	14	29[b]	29[b]	31	42[b]	73	101
SDNAI	255	23	50	177	78	261	515

[s] indicates the stool was squashed

[b] indicates the stool had bacterial over-growth

Effect of Wash Solution Volume

Each of a series of undamaged stools from one donor was washed (with a 3 min pre-soak and a 3 min wash at +/- 45° rocking angles) with both a fixed 50 ml volume of wash solution and a variable volume of wash solution based on a 0.5 ml/gram wash/stool ratio. For successive stools of the series, washing first was alternated between the fixed and variable volume. The variable volume based on the 0.5 ratio resulted on average in the use of a 64% greater volume of wash solution which visibly appeared to wet the whole stool better and produced a 21% greater DNA concentration. The second washes gave a 54% higher on average DNA yield than the first washes (largely irrespective of whether the fixed or variable wash solution volume was used first) which was presumably due to the pre-softening effect of the first washes. In general therefore the fixed ratio wash took better account of the stool weight without significantly influencing the DNA result. As shown above, stool age had an insignificant effect on the results.

stool age (days)	1	5	9	20	60	70
stool weight (g)	89	141	103	232	274	146
SDNAI from 50 ml wash	1st 55	2nd 167	1st 234	2nd 300	2nd 256	1st 49
SDNAI from 0.5 ratio wash	2nd 139	1st 92	2nd 220	1st 262	1st 207	2nd 65

Claims:

1. A method of removing cellular material from the surface of a faecal stool comprising the steps of:

a) supporting the stool in a container having a channel along which the stool extends;

b) cooling the stool to a temperature below its gel freezing point;

c) introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point; and

d) moving the container to cause the liquid to run back and forth along the channel thereby washing the cellular material from the surface of the stool.

2. A method of removing cellular material according to claim 1, wherein step d) comprises rocking the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity.

3. A method of removing cellular material according to claim 1, wherein step d) comprises displacing the container back and forth in the direction in which the channel extends.

4. A method of removing cellular material according to any one of claims 1 to 3, wherein the container has a fixed shape.

5. An apparatus for removing cellular material from the surface of a faecal stool, comprising:

mounting means for mounting a container having a channel along which, in use, the stool extends; and

motion imparting means for moving the container so that, when wash liquid is introduced into the container,

the liquid runs back and forth along the channel thereby washing cellular material from the surface of the stool.

6. An apparatus for removing cellular material according to claim 5, wherein the motion imparting means rocks the container about a horizontal axis perpendicular to the channel so that the liquid runs back and forth under gravity.

7. An apparatus for removing cellular material according to claim 5, wherein the motion imparting means displaces the container back and forth in the direction in which the channel extends.

8. An apparatus for removing cellular material according to any one of claims 5 to 7, further comprising cooling means for cooling the stool to a temperature below its gel freezing point.

9. An apparatus for removing cellular material according to any one of claims 5 to 8, further comprising filter means which filters food residues from the stool but retains the cellular material in the wash liquid when the liquid is removed from the container.

10. An apparatus for removing cellular material according to any one of claims 5 to 9, in which the container is mounted at the mounting means.

11. A container for collecting faecal stools which has a fixed shape and has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel.

12. A container according to claim 12, further having installation means for removably installing the container on a toilet with the receiving mouth opening upwardly and the collection channel extending horizontally beneath the mouth.

13. A container according to claim 11 or 12, further having cooling means for cooling a stool deposited in the container.

14. A container according to any one of claims 11 to 13, which is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein.

Fig.1a.

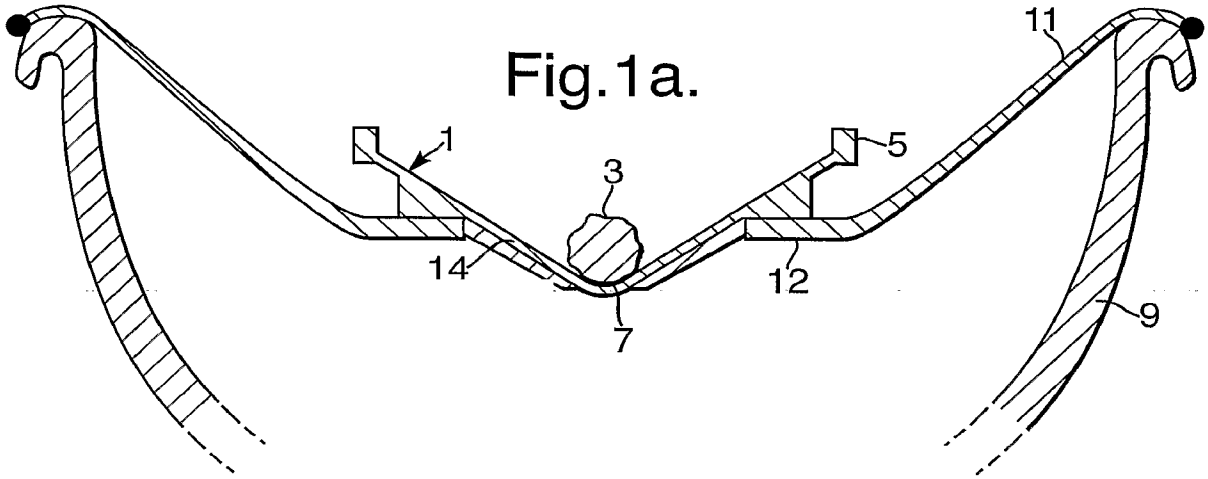


Fig.1b.

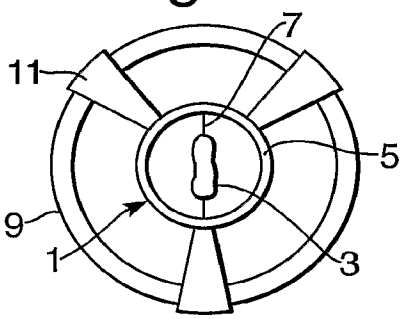


Fig.2.

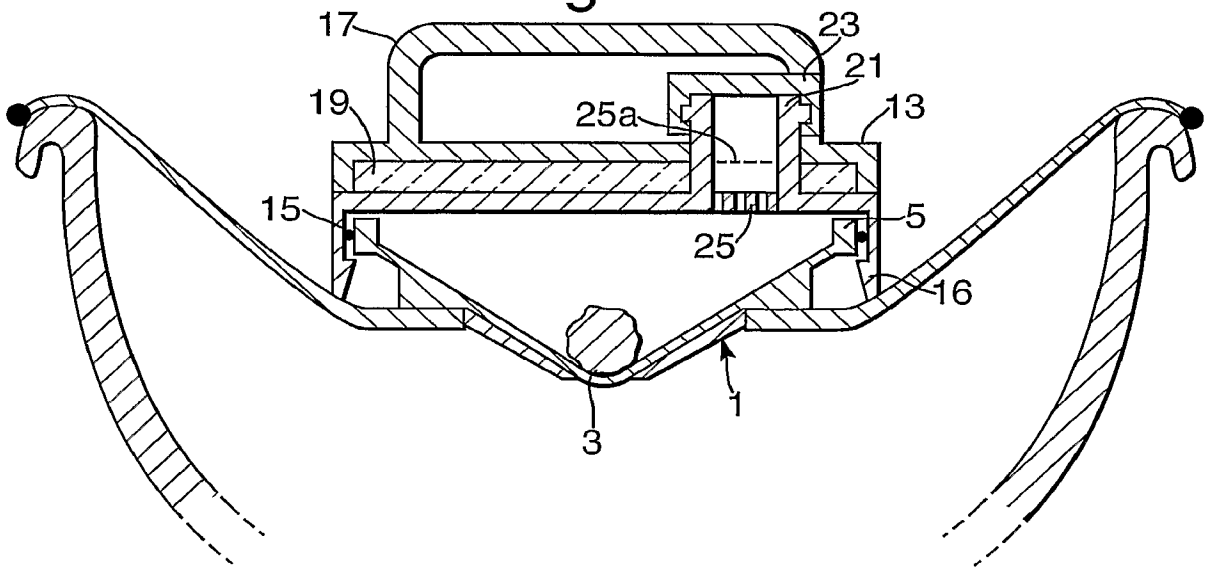


Fig.1c.

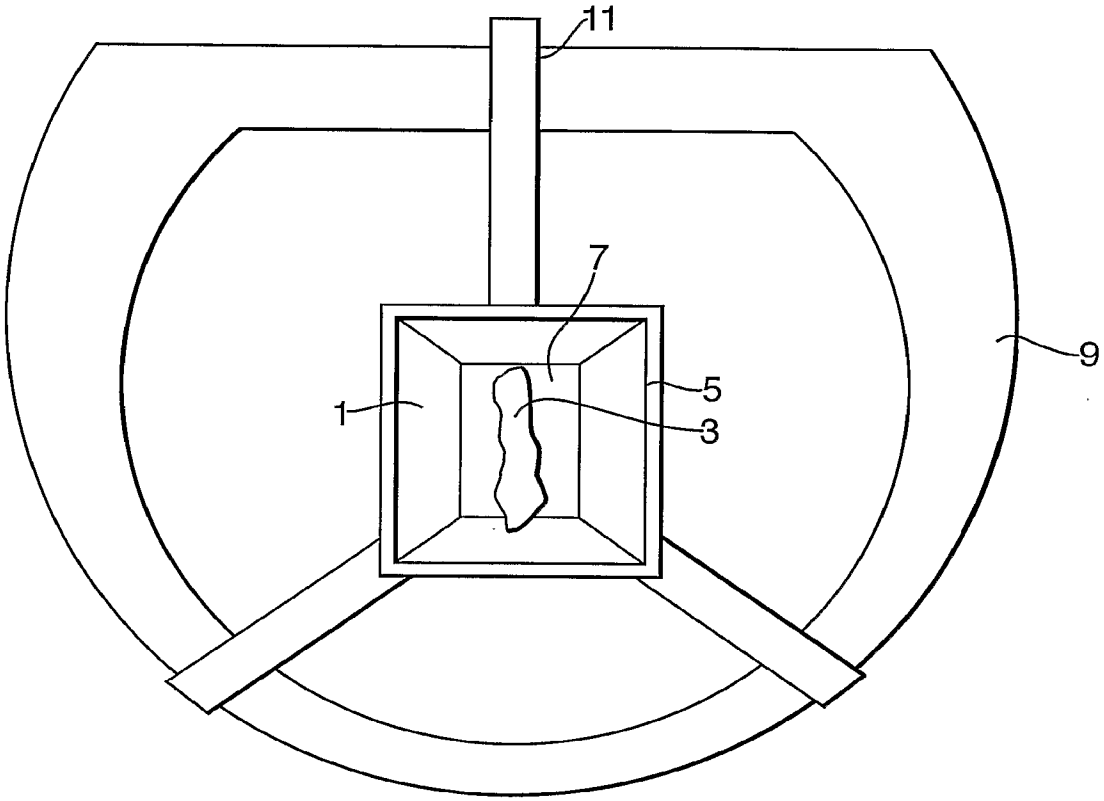


Fig.3a.

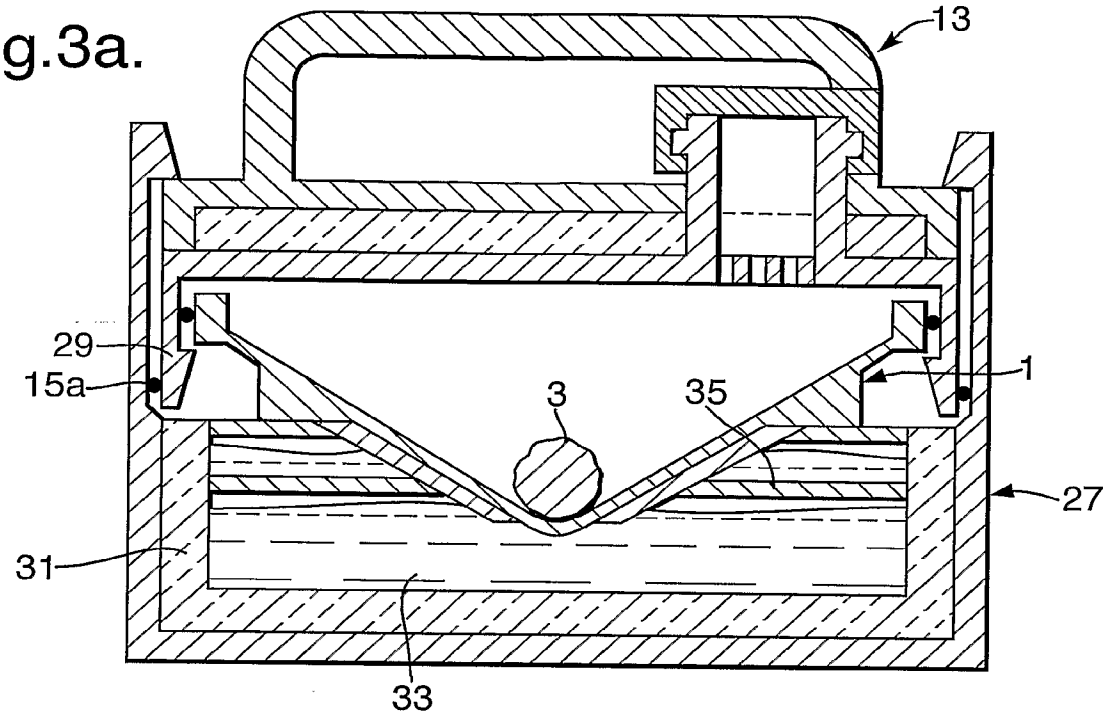

13
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Fig.3b.

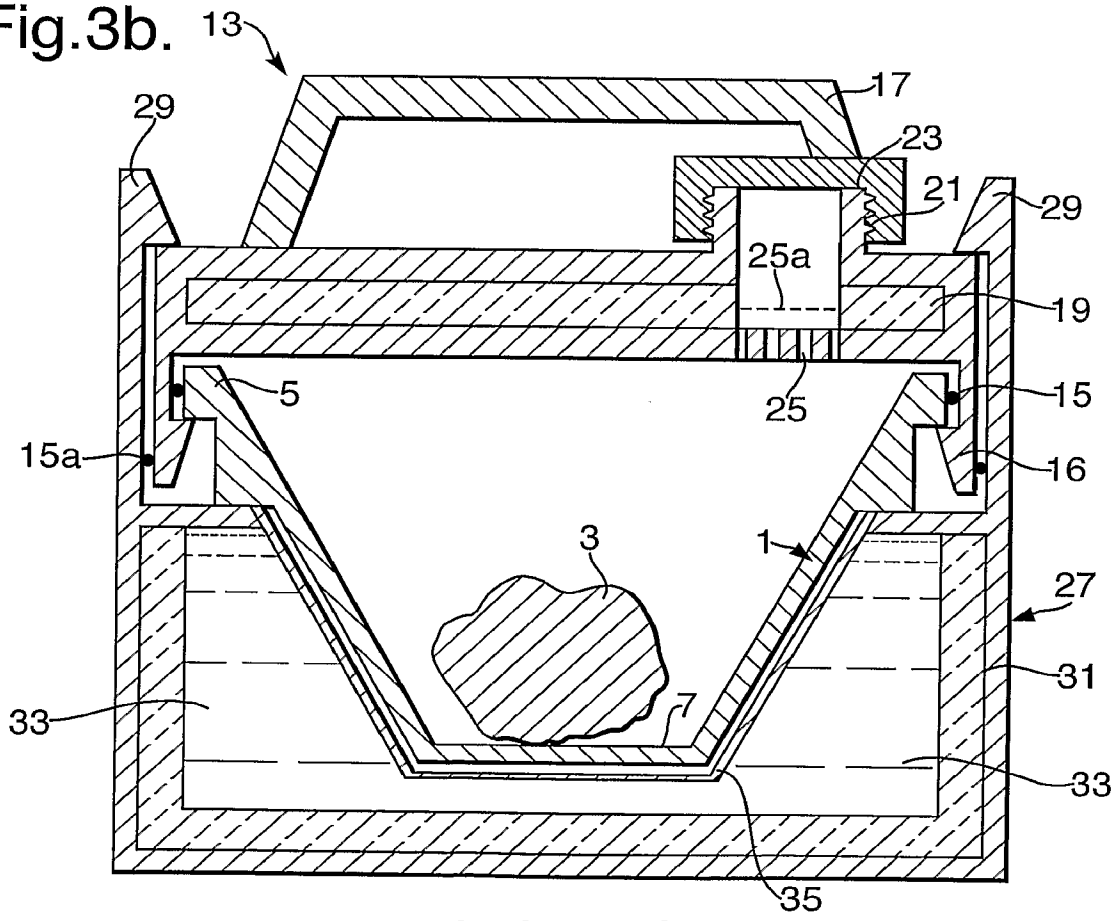

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Fig.5.

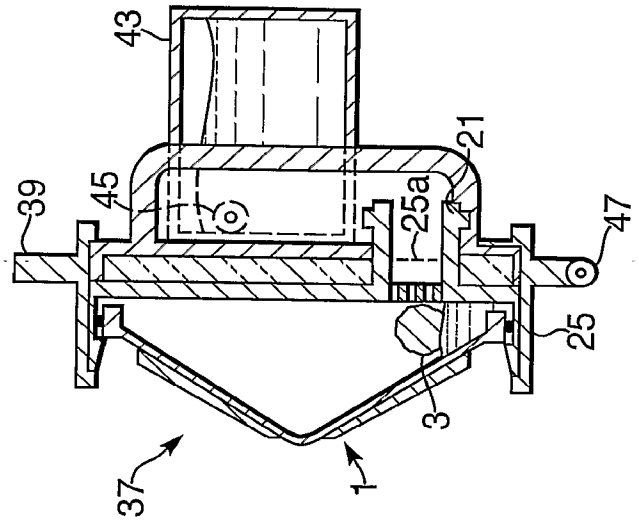
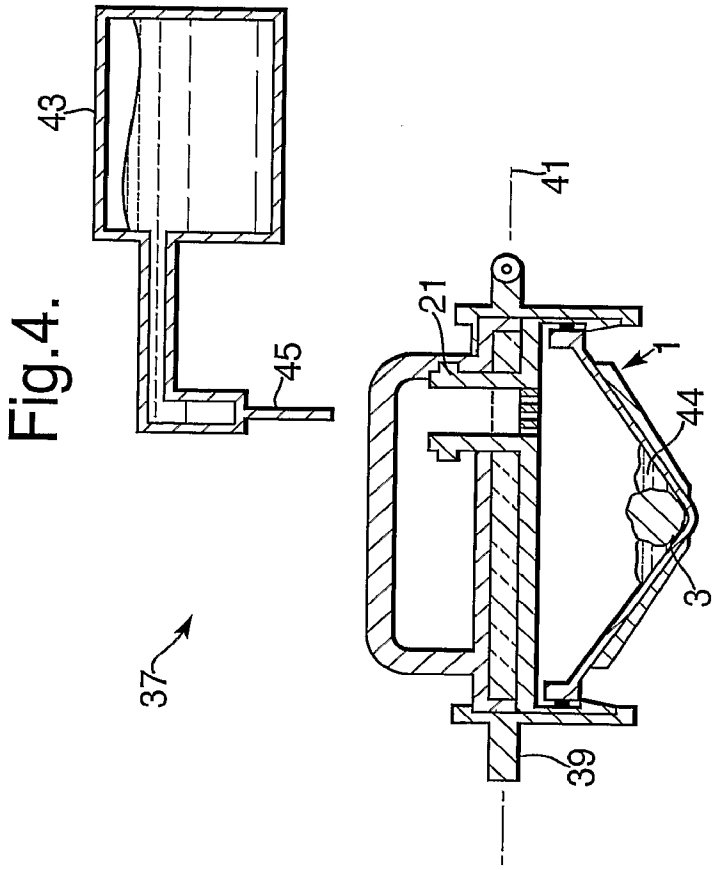


Fig.4.



INTERNATIONAL SEARCH REPORT

International Application No
B2004/003434

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01F11/00 C12Q1/68 A61B10/00 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01F C12Q A61B G01N A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 187 546 B1 (LOKTIONOV ALEXANDRE ET AL) 13 February 2001 (2001-02-13) cited in the application column 4, line 47 - column 5, line 9 column 13	1-4
X	US 2001/002985 A1 (KLEINSASSER JONATHAN) 7 June 2001 (2001-06-07) paragraphs '0052! - '0058!; figures 6,7	5,6,8,10
X	DE 15 98 515 A (GERHARDT FABRIK UND LAGER CHEM) 27 May 1971 (1971-05-27) the whole document	5,7,10
A	US 2002/119472 A1 (LAPIDUS STANLEY N ET AL) 29 August 2002 (2002-08-29) paragraph '0038!	1-4

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2004	11/11/2004

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inte ıal Application No
PCT/GB2004/003434

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 456 507 A (BRACONNIER THERESE) 12 December 1980 (1980-12-12)	11,14
Y	figure 1	13
Y	DE 199 10 836 A (KERNFORSCHUNGSANLAGE JUELICH) 21 September 2000 (2000-09-21) abstract	13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB2004/003434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6187546 B1	13-02-2001	AU 6883096 A	27-03-1997
		EP 0873502 A2	28-10-1998
		WO 9709600 A2	13-03-1997
		JP 11511982 T	19-10-1999
US 2001002985 A1	07-06-2001	AU 1574102 A	18-06-2002
		WO 0245619 A2	13-06-2002
		CA 2430758 A1	13-06-2002
DE 1598515 A	27-05-1971	DE 1598515 A1	27-05-1971
US 2002119472 A1	29-08-2002	US 6303304 B1	16-10-2001
		US 5952178 A	14-09-1999
		US 5741650 A	21-04-1998
		AU 3491999 A	01-11-1999
		CA 2328616 A1	21-10-1999
		EP 1073898 A1	07-02-2001
		JP 2002511585 T	16-04-2002
		WO 9953316 A1	21-10-1999
		AT 246806 T	15-08-2003
		AU 704696 B2	29-04-1999
		AU 2240697 A	22-08-1997
		CA 2215263 A1	07-08-1997
		DE 69629363 D1	11-09-2003
		DE 69629363 T2	09-06-2004
		DK 817968 T3	01-12-2003
		EP 1291657 A2	12-03-2003
		EP 0817968 A1	14-01-1998
		ES 2202497 T3	01-04-2004
		JP 2002515973 T	28-05-2002
		WO 9728450 A1	07-08-1997
FR 2456507 A	12-12-1980	FR 2456507 A1	12-12-1980
DE 19910836 A	21-09-2000	DE 19910836 A1	21-09-2000

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APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**06/08/2023 05:13:32 PM ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Stephanie Filandrinos
PATENT CENTER #	62233292	FILING DATE	03/07/2023
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 9

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-06-08_IDS-SB08_EXCTD-003USCON4.35239_35239.pdf	4	Information Disclosure Statement (IDS) Form (SB08)	172 KB
Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.			
OLSON_DiagnMolPathol_2005.pdf	9	Non Patent Literature	820 KB
LEUNG_AmJGastro_2007.pdf	8	Non Patent Literature	954 KB

HOEPFFNER_AlimentPharmacolTher_2005.pdf	10	Non Patent Literature	1019 KB
WO2005014154.pdf	31	Foreign Reference	1257 KB
EP1366715.pdf	9	Foreign Reference	480 KB
2023-05-22_Shuber_Decl_11634781.pdf	63	Other reference-Patent/Application/Search Documents	3205 KB
AHLQUIST_AnnInternMed_2008.pdf	23	Non Patent Literature	2103 KB
2023-05-22_Request_Reexam_11634781.pdf	286	Other reference-Patent/Application/Search Documents	11320 KB

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(71) Applicant: **Sentinel CH. S.r.L.**
20155 Milano (IT)

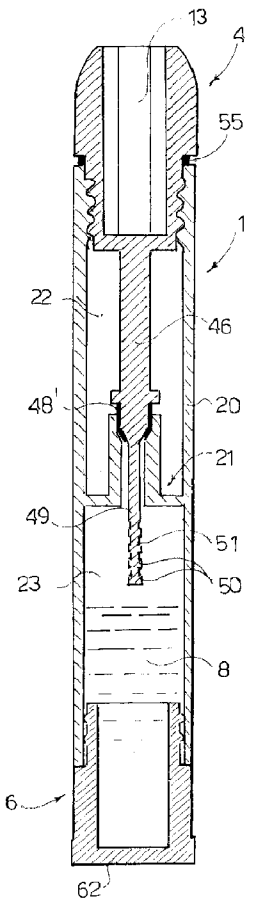
(72) Inventors:
- **De Luca, Ugo**
  20144 Milano (IT)
- **Roveda, Luigi**
  22030 Caglio (Como) (IT)
- **Fornari, Filpppo**
  20145 Milano (IT)

(74) Representative: **Petruzziello, Aldo et al**
Racheli & C. S p A
Viale San Michele del Carso, 4
20144 Milano (IT)

(54) Extraction tube for collection of faeces samples

(57) An extraction tube (1) is described for the collection of faeces samples, comprising a container body (2) hollow on the inside and open at the top to receive a top cap (4) provided with a threaded small rod (49) for collection of faeces samples. When the top cap (4) is applied to the top end of the container body (2), the threaded small rod protrudes axially into the container body. The container body (2) is also open at the bottom to receive a bottom cap (6). Said extraction tube is employed as a primary collection tube to be inserted in the sample-holder plate of automatic analysers for clinical chemistry and immunological tests.

FIG. 3



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Description

[0001] The present invention refers to an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like.

[0002] At present, the following two methods, among others, are known for testing for analytes present in faeces samples: the chemical method wherein a defined amount of the sample collected is added to a reagent constituting an active principle which reacts chemically with the analyte under examination giving rise to a chromogenic reaction indicative of the presence of the analyte and the immunological or immunochromographic method wherein the sample collected is dissolved in a buffer solution which is dropped onto a diagnostic device containing an absorbent membrane treated with specific antibodies bound to coloured substances which fix the analytes under examination giving rise to a coloured sign on the membrane.

[0003] These tests are done manually by the operator and are of a qualitative type; that is to say, they reveal the presence or absence of an analyte.

[0004] To carry out sampling of the faeces an extraction tube comprising a cylindrical container with a screw closure cap is used. In the lower inside surface of the cap there is mounted a screw small rod which protrudes axially downwards therefrom. The screw small rod has a threaded portion in its free end, destined for collection of faeces sample. In fact, when the small rod is inserted into the faeces, faeces samples are retained in its thread.

[0005] When the cap is screwed onto the mouth of the container, the screw small rod is partially immersed inside the container, which is suitably filled with a buffer solution having a suitable pH. In this manner the collected faeces sample detaches from the thread of the screw small rod and is dispersed in the buffer solution.

[0006] In the upper outside surface of the cap a breakable tip protruding axially and upward therefrom is provided. Breakage of the tip forms an outlet aperture in the cap communicating with the inside of the container. In this manner the cap acts as a dropper allowing the buffer solution - wherein faeces sample is dissolved - to drop through the outlet aperture.

[0007] During the sampling operation the patient must unscrew the cap from the container, plunge the screw small rod into the faeces so that faeces samples are retained in the thread of the screw small rod, remove with the aid of a cotton wool ball - if possible - the excess faeces accumulated on the small rod outside the thread, and lastly screw the screw cap into the container to immerse the screw small rod in the buffer solution.

[0008] Subsequently, to perform the test, the tip is broken and the buffer solution containing the sample is dropped onto a suitable diagnostic device which contains a nitrocellulose membrane treated with specific antibodies to the analyte (for example haemoglobin) under examination.

[0009] Said collection tube presents some drawbacks. In fact the operation of removing the excess faeces with cotton wool is quite awkward for the user. Furthermore, the outlet aperture of the dropper often becomes obstructed with consequent problems in releasing the solution. Also, the tip of the cap which closes the outlet aperture can break accidentally during handling and/or transport with the result of involuntary release of the solution containing biologically hazardous material.

[0010] The drawback of removal of excess faeces from the screw small rod is solved by known extraction tubes, which provide a dividing partition in the form of an annular flange disposed in the container above the level of the buffer solution. Said dividing flange has a central hole wherein the screw small rod is inserted so that the excess faeces is retained above the dividing flange and does not fall into the buffer solution.

[0011] In this case, the tip of the outlet aperture is disposed in the part of the container opposite the mouth that receives the cap of the screw small rod. Another already known solution provides a device similar to that described above wherein a second screw cap disposed to protect the tip is provided, to prevent accidental breakage thereof.

[0012] However, in extraction tubes of the prior art the problem of occlusion of the outlet aperture of the dropper nevertheless remains. Furthermore said extraction tubes have been designed for manual tests.

[0013] For use in automated systems, the buffer solution containing the dissolved faeces samples must be transferred to special sample-holder tubes which are labelled with identification codes and loaded into an automatic analyser which, in a per se known manner, performs the test.

[0014] It is apparent that said operation of transferring the buffer solution with faeces samples to the sample-holder tube of automatic analysers is quite tiresome for the operator concerned and involves an excessive waste of time.

[0015] It is an object of the present invention to overcome the prior art drawbacks by providing an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician.

[0016] Another object of the present invention is to provide an extraction and sampling tube for the collection of faeces samples that is versatile and suitable to be applied directly to different test systems present on the market, such as automatic clinical chemistry and immunological test analysers.

[0017] Yet another object of the present invention is to provide an extraction and sampling tube for faeces collection that is economical and simple to make.

[0018] These objects are achieved according to the invention with the characteristics listed in appended in-

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dependent claim 1.

[0019] Advantageous embodiments of the invention are apparent from the dependent claims. The extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body.

[0020] The main characteristic of the present invention is that said extraction tube can be used directly for automatic tests.

[0021] In the extraction tube according to the invention the breakable tip provided by the prior art extraction tubes is eliminated and, consequently, all the drawbacks due to the breakable tip are eliminated.

[0022] Furthermore, the upper part of the top cap of the extraction tube according to the invention is advantageously substantially dome-shaped, similarly to the bottom of a primary sampling tube, which makes its use possible on automatic clinical chemistry and immunological test analysers. The diameter of the container body of the extraction device according to the invention is also substantially equal to the diameter of the sample tubes routinely used in laboratories. Consequently the extraction tube according to the invention can be used directly on automatic clinical chemistry and immunological test analysers, without the need of transferring the contents thereof to special test tubes suitable to be positioned in the sample-holder plate of said automatic analysers.

[0023] Further characteristics of the invention will be made clearer by the following detailed description, referring to a purely exemplary and therefore non-limiting embodiment thereof, illustrated in the appended drawings, in which:

Figure 1 is an exploded front view illustrating the extraction tube for collection of faeces samples according to the invention;

Figure 2 is an axial sectional view along the plane of section plane II-II of Figure 1;

Figure 3 is an axial sectional view illustrating the extraction tube according to the invention assembled and with a buffer solution therein;

Figure 4 is an axial sectional view illustrating the extraction tube according to the invention, ready for use in a support of an automatic machine for clinical chemistry and immunological tests, wherein the support is shown broken off.

[0024] The extraction tube for collection of faeces samples according to the invention, designated as a whole with reference numeral 1, is described with the aid of the figures.

[0025] In particular, with reference to Figures 1 and 2 the extraction tube 1 which comprises a container 2, a top cap 4 and a bottom cap 6 is shown exploded.

[0026] The container 2 comprises a substantially cylindrical body 20, hollow on the inside and open at the top and at the bottom. Inside the body 20 of the container, in an intermediate position, a dividing partition 21 is provided which divides the inside of the container 2 into a top chamber 22 and a bottom chamber 23.

[0027] The dividing partition 21 comprises an annular flange 24 which protrudes radially inwards. A cylindrical tang 25 protrudes axially upward from the top of the annular flange 24. The dividing partition 21 has an axial through hole 26 which passes axially through the cylindrical tang 25 and the annular flange 24. The hole 26 has in its upper part a substantially tapered portion 26' with an increasing diameter with respect to the lower part.

[0028] In the inside surface of the container body 20, in the upper end of the top chamber 22, an internal thread 27 is provided.

[0029] The top cap 4 comprises a body consisting of a substantially cylindrical middle part 40 with an outside diameter substantially equal to the outside diameter of the container body 20, a substantially dome-shaped top part 41 disposed above the middle part and a substantially cylindrical bottom part 42, disposed beneath the middle part and having an outside diameter smaller than that of the middle part and slightly smaller than the inside diameter of the container body 20.

[0030] An upwardly open blind hole 43, hexagonal in shape in cross section, is provided axially in the body of the cap 4.

[0031] Knurling 44 consisting of a plurality of longitudinal ribs and grooves is provided in the outside surface of the middle part 40 of the body of the top cap 44.

[0032] An annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap.

[0033] An external thread 45 able to engage, in a screwing relationship, with the internal thread 27 of the container body is provided in the outer surface of the central bottom part 42.

[0034] A stem 46 having a smaller diameter than the bottom part 42 protrudes axially downward from the bottom part 42 of the top cap. At the bottom end of the stem 46 a collar or annular flange 47 which protrudes radially from the stem 46 is provided. The collar 47 has an outside diameter substantially equal to the outside diameter of the tang 25 of the dividing partition of the container 2.

[0035] A tang 48 with a smaller outside diameter than the collar 47 is provided beneath the collar 47. A seal 48' able engage sealably in the tapered part 26' of the hole 26 of the dividing partition 21 is disposed around the tang 48. A small rod 49 with a smaller diameter than the tang 48 and the hole 26 in the dividing partition 21 is provided axially beneath the tang 48.

[0036] A thread formed by a plurality of collars 50 disposed spaced apart from each other so as to form annular spaces 51 between said collars 50 is provided in

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the bottom portion of the small rod 49.

[0037] The bottom cap 6 comprises a body consisting of a middle cylindrical part 60 having an outside diameter substantially equal to the outside diameter of the container 2, a top cylindrical part 61 having a slightly smaller outside diameter with respect to the inside diameter of the container body 20 and a disk-shaped bottom part 62 having an outside diameter slightly greater than the outside diameter of the central part.

[0038] An upwardly open blind hole 63 is provided axially in the body of the bottom cap 6. Knurling 64 consisting of a plurality of longitudinal ribs and grooves is provided in the outer surface of the middle part 60 of the bottom cap 6.

[0039] Annular collars 65 which protrude radially outward so that the top part 61 of the bottom cap can engage by pressure in the bottom part 22 of the container are provided in the outer surface of the top part 61.

[0040] Assembly of the extraction tube 1 is described below with reference to Figure 3. The external thread 45 of the top cap 3 is screwed into the internal thread 27 of the container.

[0041] In this manner the small rod 49 of the top cap 4 enters the axial hole 26 of the dividing partition 21, until the seal 48' of the tang of the small rod settles tightly in the tapered part 26' of the hole 26 in the dividing partition 21 and at the same time the annular seal 55 of the top cap is disposed tightly on the upper edge of the container body 20. In this situation, the threaded part 50 of the small rod 49 is situated inside the bottom chamber 23 of the container, the seal 48' of the tang 48 of the small rod 49 is housed sealably inside the tapered part 26' of the hole 26 of the dividing partition and thus the top chamber 22 of the container is perfectly separated from the bottom chamber 23 of the container.

[0042] At this point a buffer solution 8 is introduced through the bottom opening of the container body 2 and then the bottom cap 6 is applied. The buffer solution 8 can be, for example, a solution with a pH between 7 and 9 with specific stabilizers. It should be noted that, by virtue of the presence of the dividing partition 21 closed by the seal 48' of the tang 48 of the stem of the top cap, the buffer solution 8 cannot pass from the bottom chamber 23 to the top chamber 22 of the container.

[0043] As shown in Figure 3, when the extraction tube 1 is in the upright position, that is to say when the bottom part 62 of the bottom cap 6 rests on a horizontal plane, the buffer solution 8 is partially inside the blind hole 63 of the bottom cap and partially in the bottom chamber 23 of the container, whilst the threaded part 50 of the small rod 49 of the top cap is not immersed in the buffer solution 8.

[0044] The user, when has to collect a faeces sample, unscrews the top cap 4 and plunges the small rod 49 into the faeces. Consequently, on extracting the small rod 49 from the faeces, faeces samples are retained in the annular spaces 51 in the thread 50 of the small rod 49, and also excess faeces remain around the small rod 49.

[0045] Subsequently the user screws the top cap 4 into the container 2. As a result the small rod 49 passes through the hole 26 in the dividing partition and the excess faeces are retained by the upper edge of the tang 25 of the dividing partition and thus remain in the top chamber 22. On the other hand, the faeces required for the analysis is retained in the annular spaces 51 of the thread 50 of the small rod.

[0046] By shaking the extraction tube 1, the buffer solution 8 immerses the threaded part 50 of the screw small rod and, as a result, the faeces samples are dispersed in the buffer solution. At this point the extraction tube 1 can be used as a primary sampling tub to be inserted in the sample-holder plate of automatic clinical chemistry and immunological test analysers.

[0047] As shown in Figure 4, in the extraction tube 1, in the upturned position, the buffer solution 8 occupies the bottom chamber 23 and totally immerses the threaded part 50 of the small rod 49. It should be noted that, even in this situation, due to the presence of the dividing partition 21 which is closed by the seal 48' of the tang of the small rod, the buffer solution 8 cannot pass from the bottom chamber 23 to the top chamber 22 of the container.

[0048] At this point the extraction tube 1 is positioned in a special housing 9 of an automatic machine for immunological tests and then the bottom cap 6 is removed so as to allow removal of the buffer solution 8 containing the faeces samples by a probe of the automatic machine.

[0049] It should be noted that the dome shape 41 of the upper part of the top cap 4 is designed to be substantially similar to the bottom of test tubes typically used in automatic analysers for clinical chemistry and immunological tests, to allow insertion of the extraction tube 1 inside the housing 9 of the automatic test machine.

[0050] Numerous changes and variations of detail within the reach of a person skilled in the art can be made to the present embodiment of the invention, without thereby departing from the scope of the invention as set forth in the appended claims.

Claims

1. An extraction tube (1) for collection of faeces samples comprising:

   - a container body (2), hollow on the inside, open at the top, and able to receive a buffer solution,
   - a top cap (4) provided with a threaded small rod (49) for collection of faeces samples, said threaded small rod protruding axially inside the container body, when the top cap (4) is applied to the top end of the container body (2),
   - a dividing partition (21) provided, in an interme-

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diate position, inside said container body (2) so as to separate a top chamber (22) from a bottom chamber (23) inside said container body, said dividing partition (21) having an axial hole (26) suitable to allow the passage of said threaded small rod (49), so as to retain the excess faeces in said top chamber (22) and allow the passage of the threaded part of the small rod into said bottom chamber (23),

characterised in that said container body is open at the bottom and provided with a bottom cap (6) which can be applied movably to the bottom end of the container body, so that said extraction tube can be used directly as a primary sampling tube to be inserted in a sample-holder plate of automatic analysers, following removal of said bottom cap (6) and overturning of said container body (2) for positioning in a housing (9) of the automatic analyser, with the top cap (4) facing downward.

2. An extraction tube (1) according to claim 1, **characterised in that** said top cap (4) has an upper portion (41) destined to be disposed outside said container body (2), said upper portion (41) having a substantially domed shape similar to the bottom of a test tube normally used in the sample-holder plate of automatic analysers.

3. An extraction tube according to claim 1 or 2, **characterised in that** said top cap (4) comprises a stem (46) ending in an annular flange (47) beneath which is provided a cylindrical tang (48) surrounded by a seal (48') beneath which said threaded small rod (49) protrudes axially, and said dividing partition (21) comprising an annular flange (24) from which a cylindrical tang (25) protrudes axially upward so that when said top cap (4) is applied to the top end of the container body, the seal 48' of the cylindrical tang (48) of the stem (46) of the top cap is housed sealably inside a tapered part (26') of said hole (26) in the dividing partition.

4. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said top cap (4) comprises a bottom portion (42) having an external thread (45) able to engage in an internal thread (27) provided in the top inner part of said container body (2).

5. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said top cap (4) comprises a middle portion (40) having knurling (42) consisting of a plurality of longitudinal ribs and grooves.

6. An extraction tube (1) according to claims 4 and 5, **characterised in that** between said knurled part (44) of the top cap and said threaded part (45) of the top cap there is provided an annular seal (55) able to abut sealably on the top edge of said container body 2.

7. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said top cap (4) has a blind hole (43) axially open at the top to the outside of the container body, and hexagonal in shape in cross section.

8. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said bottom cap (6) has a top portion (61) with a plurality of annular collars (65) protruding radially outwards, to be able to be applied by pressure into the bottom part of said container body (2).

9. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said bottom cap (6) comprises a middle portion (60) with knurling (64) consisting of a plurality of longitudinal ribs and grooves.

10. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said bottom cap (6) has a blind hole (63) axially open at the top to the inside of the container body (2).

11. An extraction tube (1) according to any one of the preceding claims, **characterised in that** said threaded small rod (49) has in its bottom portion a thread consisting of a plurality of concentric collars (50) so as to define a plurality of annular spaces (51) therebetween, able to retain the sampled faeces.

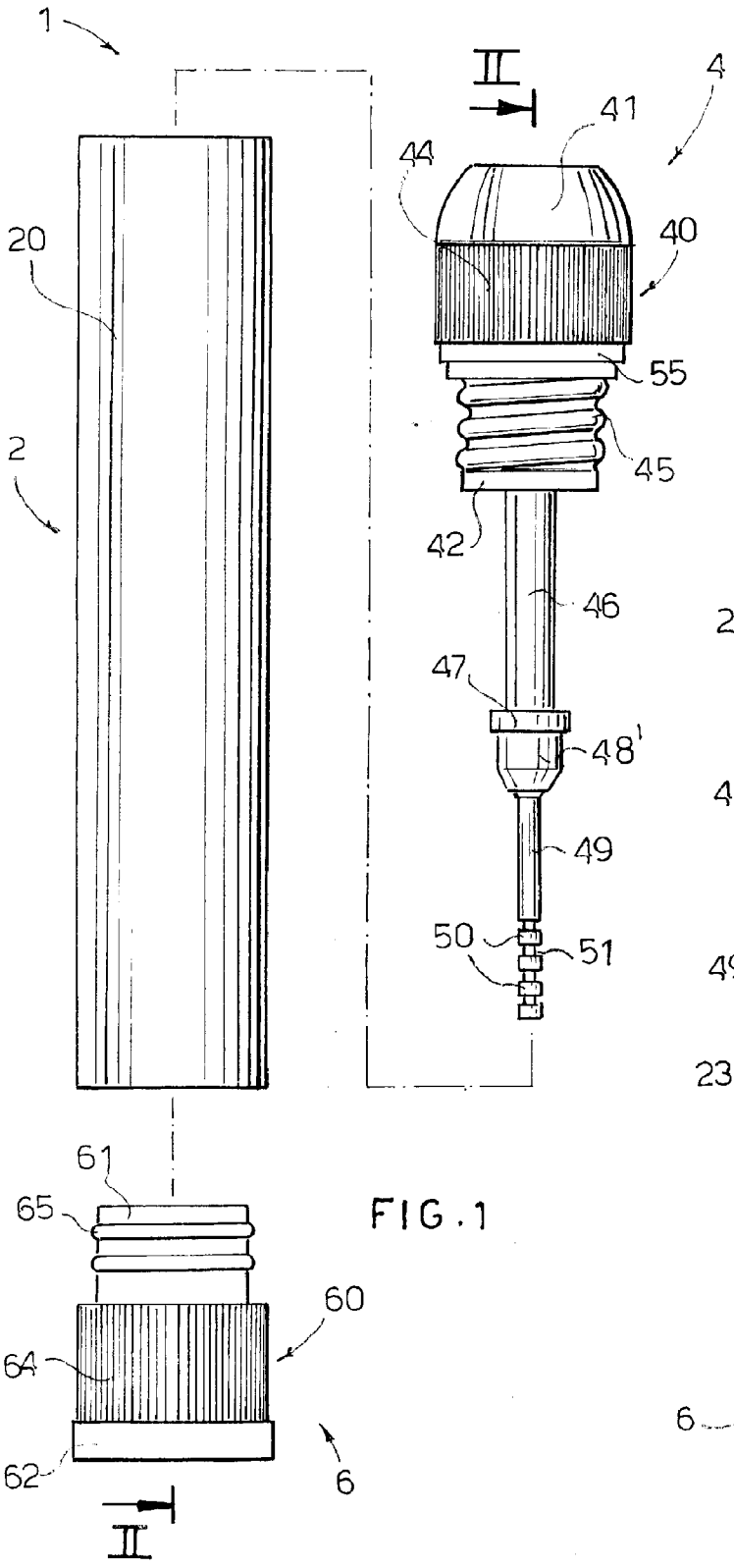

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FIG. 1

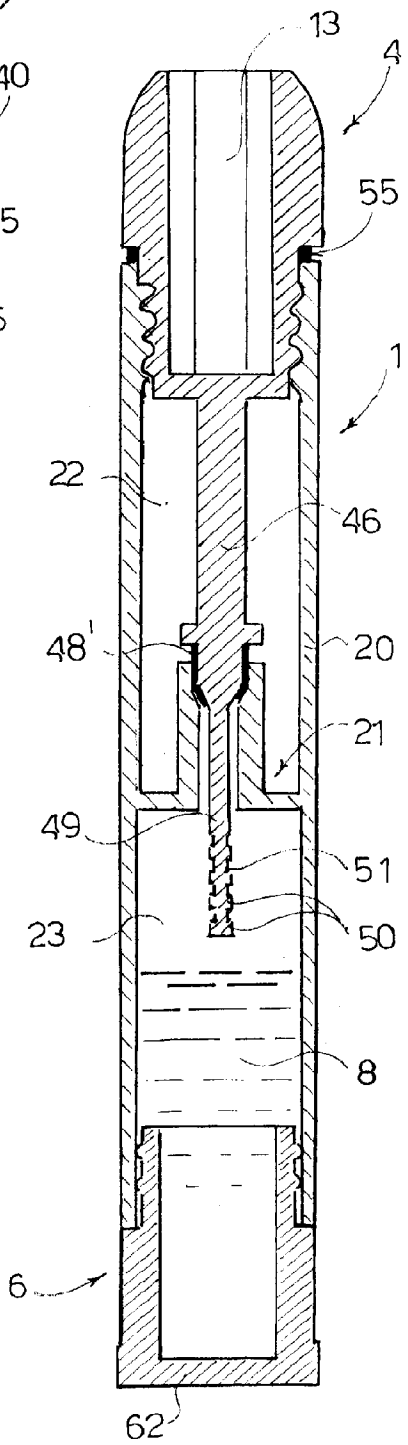

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FIG. 3

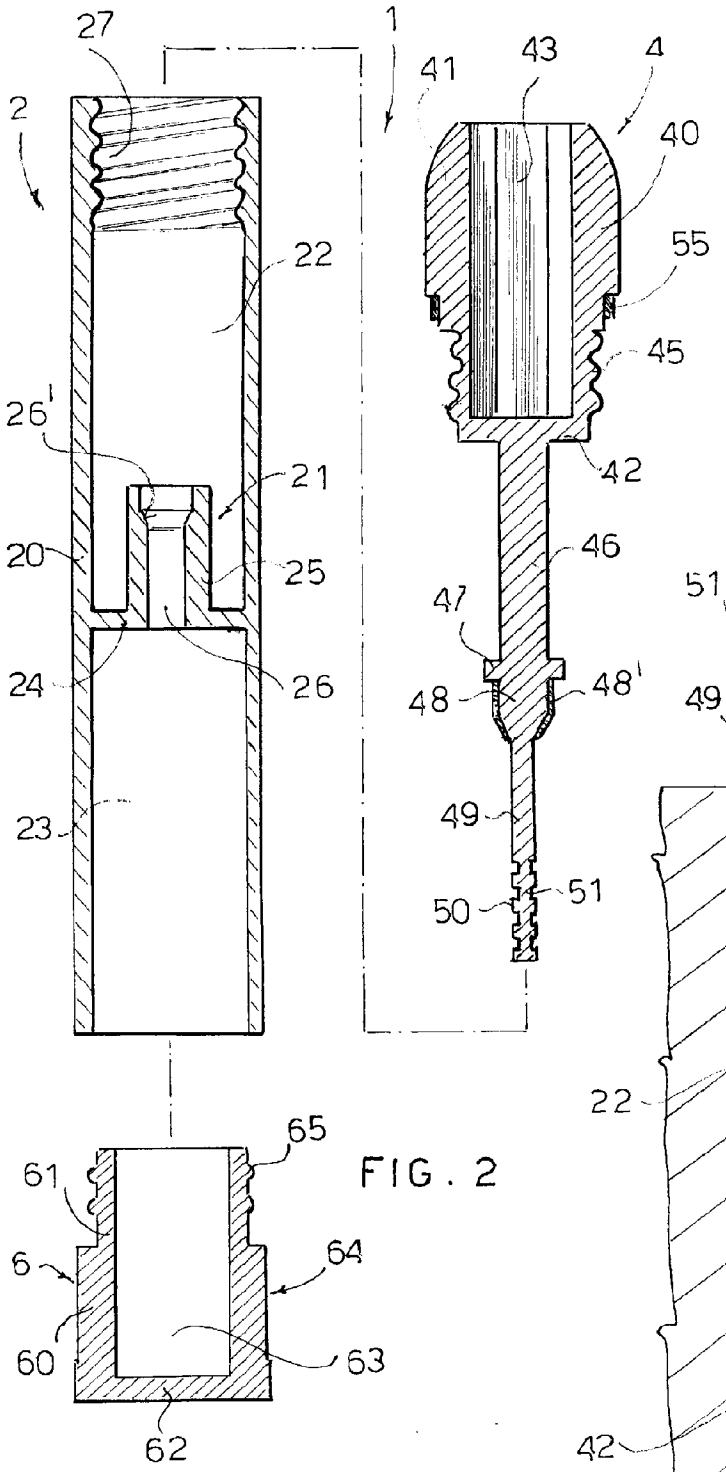

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FIG. 2

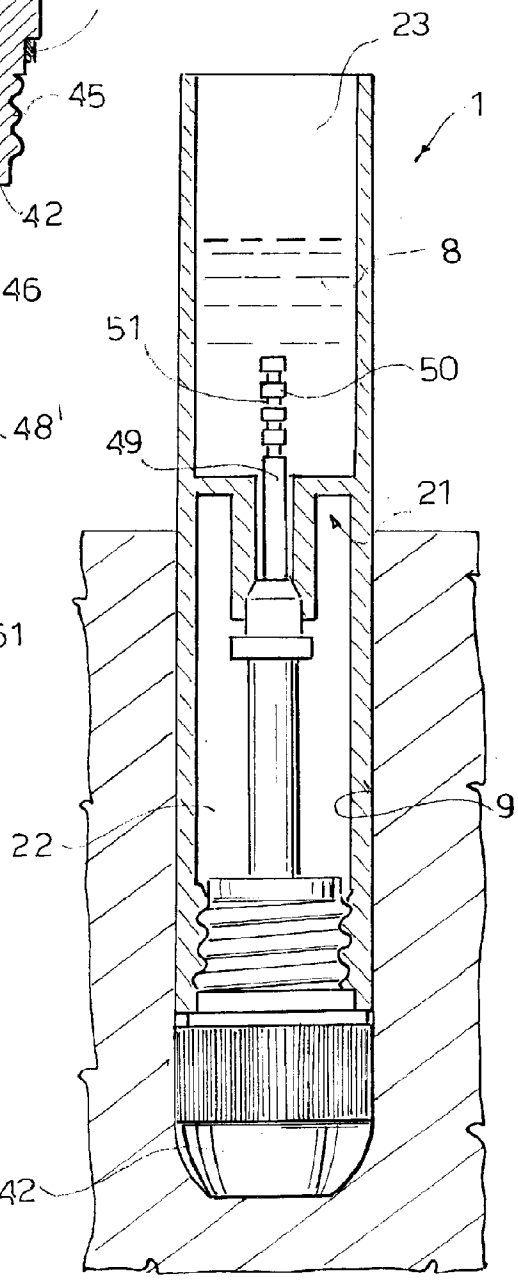

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FIG. 4

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 01 1896

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	EP 0 727 653 A (WAKO PURE CHEM IND LTD) 21 August 1996 (1996-08-21) * column 3, line 17 - line 38 * * column 4, line 58 - column 5, line 6 * * figure 1 * ---	1	A61B10/00 B01L3/14 B01L3/00
A	US 6 299 842 B1 (KOZAK KENNETH JAMES ET AL) 9 October 2001 (2001-10-09) * column 3, line 53 - column 4, line 13 * * figure 1 * -----	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61B
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G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21 July 2003	Compos, F

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21-07-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0727653 A	21-08-1996	DE 69610569 D1	16-11-2000
		DE 69610569 T2	31-05-2001
		EP 0727653 A2	21-08-1996
		ES 2151090 T3	16-12-2000
		JP 8285845 A	01-11-1996
		US 6207113 B1	27-03-2001
		US 5882942 A	16-03-1999
US 6299842 B1	09-10-2001	AU 3727200 A	21-09-2000
		WO 0051496 A1	08-09-2000
		US 2002007686 A1	24-01-2002

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

EXAMINER	
CENTRAL, DOCKET	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
06/05/2023	ELECTRONIC

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

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

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

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

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 18/179,945	**Applicant(s)** Louwagie, Joost	
	Examiner KIMBERLY A INABINET	**Art Unit** OPET	**AIA (FITF) Status** No

1. THE REQUEST FILED 07 March 2023 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to KIMBERLY INABINET at (571) 272-4618. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/KIMBERLY A INABINET/ Paralegal Specialist, OPET	

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
18/179,945	03/07/2023		1820	EXCTD-35239.307	20	1

CONFIRMATION NO. 2966

173254
CASIMIR JONES, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

FILING RECEIPT

OC000000057308692

Date Mailed: 05/22/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Joost Louwagie, Dornach, SWITZERLAND;

Applicant(s)

Exact Sciences Corporation, Madison, WI;

Power of Attorney: The patent practitioners associated with Customer Number 173254

Domestic Priority data as claimed by applicant

This application is a CON of 17/936,335 09/28/2022 PAT 11,634,781
which is a CON of 15/634,607 06/27/2017
which is a CON of 15/010,436 01/29/2016 ABN *
which is a CON of 13/147,570 03/12/2012 ABN
which is a 371 of PCT/GB2010/000180 02/03/2010
which claims benefit of 61/149,581 02/03/2009

(*) Data provided by applicant is not consistent with PTO records.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 05/19/2023

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/179,945**

Projected Publication Date: 08/31/2023

Non-Publication Request: No

Early Publication Request: No

Title

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number
18/179,945

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	320
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	700
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	800
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	* 0			OR	x =	0
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x =	0
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1820

APPLICATION AS AMENDED - PART II

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

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

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

Doc Code: PAT.
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 03/31/2021. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
18/179,945	07-Mar-2023

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 173254

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

EXACT SCIENCES CORPORATION

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Jennifer M. Duke/	Date (Optional)	
Name	Jennifer M. Duke		
Title	Sr. Director, Lead Counsel Intellectual Property		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

Privacy Act Statement

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

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

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

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**04/20/2023 03:43:32 PM ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Jeremiah Gile
PATENT CENTER #	61957225	FILING DATE	-
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-03-07_35239-307_ExecPOA.pdf	2	Power of Attorney	162 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized

by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

Application Number: 18179945

Reviewed by: kBuLmOjFP3VzlESORWEnm76I/npXqkJwHo0JQ1qYXov9ZBL4CQRFMQ==

Note: This validation report concerns the sequence listing filed 03-07-2023 18:07:59 and indicates that NO errors were found by the USPTO during validation of the sequence listing. This validation report should not be construed as meaning that the sequence listing complies with all of the requirements of 37 CFR §§ 1.821-1.825, 37 CFR §§ 1.831-1.835 and 1.839, WIPO Standard ST.25, or WIPO Standard ST.26.

For any sequence listing related questions or concerns, the Applicant is invited to contact the USPTO Sequence Listing Help Desk at 571-272-2510 or SequenceHelpDesk@USPTO.GOV.

Timestamp: [year=2023; month=3; day=8; hr=8; min=34; sec=47; ms=191]

Validated By CRFValidator v 1.5.0

Application No: 18179945 Version No: 1.1

CRF Copy Request from application Version :

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Actual SeqID Count: 39

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				Application Number	
				Filing Date	
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	1	of	5	Attorney Docket Number	EXCTD-35239.307

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4437975	1984-03-20	GILLESPIE et al.	
		4445235	1984-05-01	SLOVER et al.	
		4582811	1986-04-15	PUCCI et al.	
		4683197	1987-07-28	GALLATI	
		4889818	1989-12-26	GELFAND et al.	
		5198365	1993-03-30	GROW et al.	
		6410276	2002-06-25	BURG et al.	
		6761702	2004-07-13	SMITH	
		7288413	2007-10-30	GOULDEN	
		7371527	2008-05-13	BAYLIN et al.	
		8969046	2015-05-03	VAN ENGELAND et al.	

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20020187476	2002-12-12	KOROULIS et al.	
		20020096469	2002-07-25	FAULKNER	
		20030086869	2003-05-08	STALLINGS	
		20030096244	2003-05-22	RABELLO et al.	
		20040019298	2004-01-29	ZHOU et al.	
		20040091881	2004-05-13	OLEK et al.	
		20050064401	2005-03-24	OLEK et al.	
		20050075543	2005-04-07	CALABRESE	
		20050244836	2005-11-03	TSANG	
		20060084054	2006-04-20	ALSOBROOK et al.	
		20060188939	2006-08-24	GAO	
		20060210448	2006-09-21	WANG et al.	

Examiner Signature		Date Considered	

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				First Named Inventor	JOOST LOUWAGIE
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				Examiner Name	
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U.S. PUBLISHED PATENT APPLICATIONS					
		20060216830	2006-09-28	KIKUIRI	
		20070017015	2007-01-25	FINELL	
		20070072214	2007-03-29	GARVIN	
		20080097238	2008-04-24	LOKTIONOV et al.	
		20080124714	2008-05-29	SHUBER et al.	
		20080221056	2008-09-11	BAYLIN et al.	
		20080227208	2008-09-18	YEE et al.	
		20090004058	2009-01-01	LIANG et al.	
		20100136572	2010-06-03	ATAMAN-ONAL et al.	
		20120164238	2012-06-28	LOUWAGIE	
		20160194723	2016-07-07	LOUWAGIE	
		20230048152	2023-02-16	LOUWAGIE	

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FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] *(if known)*	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 0032782	1981-07-29	KOMMANDIITTIYHTIO FINNPIPETTE		
		EP 0308227	1989-03-22	SMITHKLINE DIAGNOSTICS, INC.		
		EP 0817968	1998-01-14	EXACT SCIENCES CORPORATION		
		FR 2919065	2009-01-23	BIOMERIEUX		
		WO 1990/06995	1990-06-28	SISKA DIAGNOSITCS, INC.		
		WO 1997/46705	1997-12-11	THE JOHNS HOPKINS SCHOOL OF MEDICINE		
		WO 2000/050640	2000-08-31	EXACT LABORATORIES, INC.		
		WO 2004/067726	2004-08-12	KECK GRADUATE INSTITUTE		

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				Application Number	
				Filing Date	
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	3	of	5	Attorney Docket Number	EXCTD-35239.307

FOREIGN PATENT DOCUMENTS						
		WO 2004/083399	2004-09-30	THE JOHNS HOPKINS UNIVERSITY.		
		WO 2004/0927209	2004-10-28	KIETURAKIS & KARCZEWSKI		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		
		WO 2005/017207	2005-02-24	CASE WESTERN RESERVE UNIVERSITY		
		WO 2006/113671	2006-10-26	ONCOMETHYLOME SCIENCES SA		
		WO 2008/010975	2008-01-24	THE JOHNS HOPKINS UNIVERSITY		
		WO 2008/084219	2008-07-17	ONCOMETHYLOME SCIENCES SA		
		WO 2008/100913	2008-08-21	THE JOHNS HOPKINS UNIVERSITY		
		WO 2008/102002	2008-08-28	RIKSHOSPITALET RADIUMHOSPITALE		

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
		ABBASZADEGAN et al., Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran. World J Gastroenterol. 2007 Mar 14;13(10):1528-33	
		AHLQUIST et al., Cologuard Primed to Change Landscape of CRC Screenin", Mayo Clinic Clinical Updates, http://www.mayoclinic.org/medical-professionals/clinical-updates/digestivediseases/ cologuard-primed-to-change-landscape-of-crc-screening, Pages 1-4, 12/03/2014.	
		AUERKARI, Methylation of tumor suppressor genes p16(INK4a), p27(Kip1) and E-cadherin in carcinogenesis. Oral Oncol. 2006 Jan;42(1):5-13	
		CHEN et al., Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. J Natl Cancer Inst. 2005 Aug 3;97(15):1124-32	
		CONROY et al., Exact Sciences Completes 40,000 Cologuard Tests During First Quarter 2016, Exact Sciences Latest News, Pages 1-10, 05/03/2016.	

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	4	of	5	Attorney Docket Number	EXCTD-35239.307

NONPATENT LITERATURE DOCUMENTS			
		EXACT SCIENCES, Cologuard(TM) sDNA-based Colorectal Cancer Screening Test - Instructions for Use, Pages 1-71, 2013.	
		HAMMER, Human hybirds. Sci Am. 2013 May;308(5):66-71	
		ITZKOWITZ et al., A simplified, noninvasive stool DNA test for colorectal cancer detection. Am J Gastroenterol. 2008 Nov;103(11):2862-70	
		KARL et al., Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. Clin Gastroenterol Hepatol. 2008 Oct;6(10):1122-8	
		KIM et al., Noninvasive Molecular Biomarkers for the Detection of Colorectal Cancer. BMB Rep. 2008 Oct 31;41(10):685-92	
		KUTZNER et al., Non-invasive detection of colorectal tumours by the combined application of molecular diagnosis and the faecal occult blood test. Cancer Lett. 2005 Nov 8;229(1):33-41	
		LAIRD, The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003 Apr;3(4):253-66	
		LENHARD et al., Analysis of promoter methylation in stool: a novel method for the detection of colorectal cancer. Clin Gastroenterol Hepatol. 2005 Feb;3(2):142-9	
		LEVI et al., A quantitative immunochemical faecal occult blood test is more efficient for detecting significant colorectal neoplasia than a sensitive guaiac test. Aliment Pharmacol Ther. 2006 May 1;23(9):1359-64	
		OHLSSON et al., Biomarker selection for detection of occult tumour cells in lymph nodes of colorectal cancer patients using real-time quantitative RT-PCR. Br J Cancer. 2006 Jul 17;95(2):218-25	
		MULDER et al., Tumor pyruvate kinase isoenzyme type M2 and immunochemical fecal occult blood test: performance in screening for colorectal cancer. Eur J Gastroenterol Hepatol. 2007 Oct;19(10):878-82	
		REIN et al., Identifying 5-methylcytosine and related modifications in DNA genomes. Nucleic Acids Res. 1998 May 15;26(10):2255-64	
		SHASTRI et al., Comparison of an established simple office-based immunological FOBT with fecal tumor pyruvate kinase type M2 (M2-PK) for colorectal cancer screening: prospective multicenter study. Am J Gastroenterol. 2008 Jun;103(6):1496-504	
		SOMMER et al., Minimal homology requirements for PCR primers. Nucleic Acids Res. 1989 Aug 25;17(16):6749	
		TURGEON et al., Fecal DNA-Based Detection of Colorectal Neoplasia. Curr Colorectal Cancer Rep. 2007 Oct;3(4):171-177	

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	5	of	5	Attorney Docket Number	EXCTD-35239.307

NONPATENT LITERATURE DOCUMENTS			
		VILLAR-GAREA et al., DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Curr Drug Metab. 2003 Feb;4(1):11-31	

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	JOOST LOUWAGIE
				Art Unit	
				Examiner Name	
Sheet	6	of	5	Attorney Docket Number	EXCTD-35239.307

CERTIFICATION STATEMENT

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

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

OR

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

☐ See attached certification statement.

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

☒ A certification statement is not submitted herewith.

☒ Documents not provided herewith have been previously cited in parent U.S. patent application number 13/147,570 filed on March 12, 2012; 15/010,436, filed on January 29, 2016; 15/634,607 filed on June 27, 2017; and/or 17/936,335 filed on September 28, 2022. In compliance with 37 C.F.R. § 1.98(d), Applicants have not included copies of these documents.

SIGNATURE

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

Signature	/Mary Ann D. Brow/	Date (YYYY-MM-DD)	2023-03-07
Name/Print	Mary Ann D. Brow	Registration Number	42363

.

PTO Notes regarding this form:

[1] Applicant's unique citation designation number (optional).
[2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.

[3] Applicant's unique citation designation number (optional).
[4] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.
[5] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).
[6] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
[7] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.
[8] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**.

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	03/07/2023 06:07:59 PM ET	EXCTD-35239.307

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Stephanie Filandrinos
PATENT CENTER #	61703441	AUTHORIZED BY	Mary Ann Brow
CUSTOMER #	173254	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Joost Louwagie

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 0638	E202337I08235000	Stephanie Filandrinos

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	140.00	1	140.00
1817	REQUEST FOR PRIORITIZED EXAMINATION	4200.00	1	4200.00
1111	UTILITY PATENT APPL. SEARCH FEE	700.00	1	700.00
1011	BASIC FILING FEE - UTILITY (PAPER FILING ALSO REQUIRES NON-ELECTRONIC FILING FEE UNDER 1.16(T))	320.00	1	320.00
1311	PATENT APPL. EXAMINATION FEE	800.00	1	800.00

TOTAL AMOUNT:	$6,160.00

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Methods of Detecting Colorectal Cancer

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 15/010,436 filed on 29-Jan-2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Joost Louwagie Date (Optional): 23 Jan 2016

Signature: [signature]

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

uspto UNITED STATES PATENT AND TRADEMARK OFFICE

PTO/AIA/14
OMB 0651-0032

Application Data Sheet 37 CFR 1.76

The Application Data Sheet is part of the provisional or non-provisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.

Inventor Information

of inventors: 1

1. **Joost Louwagie**

Residence Information
Non-US Residency
Dornach
SWITZERLAND

Mailing Address
c/o Exact Sciences Corporation, 5505 Endeavor Lane
Madison, WI 53719
UNITED STATES

Application Information

Customer number	173254 -
Correspondence address	---
Title of invention	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD
Attorney docket number	EXCTD-35239.307
Entity status	Regular Undiscounted
Application type	Nonprovisional Application under 35 USC 111(a)

Subject matter	Utility
Total number of drawing sheets	---
Suggested figure for publication	---
Filing by reference	No
Publication request	Normal eighteen-month publication

Representative Information

of representatives: 1

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).

Customer number 173254

Domestic Benefit/National Stage Information

of benefit claims: 6

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c), 386(c), or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior app status	Pending
Application number	---
Continuity type	**Continuation of**
Prior app number	**17936335**
Filing Date	09/28/2022

Prior app status	Pending
Application number	**17936335**
Continuity type	**Continuation of**

Prior app number	**15634607**
Filing Date	06/27/2017

Prior app status	Abandoned
Application number	**15634607**
Continuity type	**Continuation of**
Prior app number	**15010436**
Filing Date	01/26/2016

Prior app status	Abandoned
Application number	**15010436**
Continuity type	**Continuation of**
Prior app number	**13147570**
Filing Date	03/12/2012

Prior app status	Expired
Application number	**13147570**
Continuity type	**A 371 of international**
Prior app number	**PCT/GB2010/000180**
Filing Date	02/03/2010

Prior app status	Expired
Application number	**PCT/GB2010/000180**
Continuity type	**Claims benefit of provisional**
Prior app number	**61149581**
Filing Date	02/03/2009

Foreign Priority Information

of foreign priority claims: 1

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the Application Data Sheet constitutes the claim for priority as required by 35 U.S.C. 119 (b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified

copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application number	PCT/GB2010/000180
Country	WO
Filing Date	02/03/2010
Access code	---

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

Checking this box will cause the application to be examined under the first inventor to file provisions of the AIA.

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 2016, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization or Opt-Out of Authorization to Permit Access

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

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

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

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

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

bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h) (1).

B. **Search Results from U.S. Application to EPO**

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

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

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

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

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

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

Applicant Information

of applicants: 1

The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46.

1. **Exact Sciences Corporation**
5505 Endeavor Lane
Madison, WI 53719
UNITED STATES

Phone: ---
Fax: ---
Email: ---

Applicant Organization
Applicant type Assignee

Assignee Information including Non-Applicant Assignee Information

of assignees: 0

An assignee-applicant identified in the "Applicant" section will appear on the patent application as an applicant.

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

Data was not provided for this section.

Signature

NOTE:

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

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

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

Signature	First name	Last name	Registration #	Date
/Mary Ann D. Brow/	Mary Ann D.	Brow	42363	03/07/2023

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/179,945	**03/07/2023 06:07:59 PM ET**	**EXCTD-35239.307**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	2966	FILED BY	Stephanie Filandrinos
PATENT CENTER #	61703441	FILING DATE	-
CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost Louwagie
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Mary Ann Brow

Documents

TOTAL DOCUMENTS: 8

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
generatedADS61703441.pdf	6	Application Data Sheet	138 KB
2023-03-07_35239-307_TrackOne_Form.pdf	2	Track One Request	117 KB
35239US4CON_Exec_Decl.pdf	1	Oath or Declaration filed	106 KB
Warning: The attached file contains comments or annotations. Comments are not recommended and may cause processing problems with the document.			
35239-307_SEQUENCE_LISTING.xml	-	Sequence Listing (ST.26 XML File)	35 KB

2023-03-07_APPLICATION_EXCTD-003USCON4.35239_35239.pdf		62	-	577 KB
2023-03-07_APPLICATION_EXCTD-003USCON4.35239_35239-SPEC.pdf	(1-57)	57	Specification	569 KB
2023-03-07_APPLICATION_EXCTD-003USCON4.35239_35239-CLM.pdf	(58-61)	4	Claims	140 KB
2023-03-07_APPLICATION_EXCTD-003USCON4.35239_35239-ABST.pdf	(62-62)	1	Abstract	107 KB
2023-03-07_35239-307_IDS-SB082.pdf		7	Information Disclosure Statement (IDS) Form (SB08)	196 KB

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Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
generatedADS61703441.pdf	BD4C841F514981C84CF1231FFE6621D842B0FAB4C8F090ED7A8CF0ACC39FBFEA07BAB6EE7FC6CD9BF421184567512B81E5E1852677D8D400FEAE0B8783974A1A
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New Applications Under 35 U.S.C. 111

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

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

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage

submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Joost Louwagie	Nonprovisional Application Number (if known):	
Title of Invention:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Mary Ann D. Brow/	Date 2023-03-07
Name (Print/Typed) Mary Ann D. Brow	Practitioner Registration Number 42363

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application No. 17/936,335, filed September 28, 2022, now allowed, which is a continuation of U.S. Patent Application No. 15/634,607, filed June 27, 2017, which is a continuation of U.S. Application No. 15/010,436, filed January 29, 2016, now abandoned, which is a continuation of U.S. Application No. 13/147,570, filed March 12, 2012, now abandoned, which is the U.S. national stage of International Application PCT/GB2010/000180, filed February 3, 2010, which claims priority to U.S. Provisional Application No. 61/149,581, filed February 3, 2009. The contents of these applications are incorporated herein by reference in their entireties.

SEQUENCE LISTING

The text of the computer readable sequence listing filed herewith, titled "35239-307_SEQUENCE_LISTING", created March 6, 2023, having a file size of 35,640 bytes, is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods and kits for analysis of fecal samples comprising removing a portion of a collected fecal sample and adding the removed portion of the fecal sample to a buffer that prevents denaturation or degradation of blood proteins found in the sample, and comprising detection of blood in the removed portion of the fecal sample, *e.g.*, by immunoassay.

BACKGROUND OF THE INVENTION

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, and is the second leading cause of cancer-related deaths in the United States. A patient's prognosis is good if the cancer is caught early, when the site of the cancer is confined to its site of

origin. However, the cure rates fall once the cancer has spread. Most colon cancers arise from conventional adenomatous polyps (conventional adenoma-to-carcinoma sequence), while some colon cancers appear to arise from the recently recognized serrated adenomatous polyp (serrated adenoma-to-carcinoma theory). Because conventional adenomas and serrated adenomas are usually asymptomatic, mass screening of asymptomatic patients has become the cornerstone for detecting and eliminating these precursor lesions to reduce the risk of colon cancer.

A number of different screening methods for CRC are available. Procedures such as digital rectal examination (DRE); colonoscopy or sigmoidoscopy are highly invasive, painful and can cause a great deal of patient discomfort. Other less invasive screening tests include fecal occult blood test (FOBT); fecal immunochemical test (FIT); barium enema with air contrast; virtual colonoscopy; biopsy (e.g., CT guided needle biopsy); and imaging techniques (e.g., ultrasound, CT scan, PET scan, and MRI).

Colonoscopy has become the primary screening test for CRC because of its high sensitivity and specificity, and the ability to perform polypectomy. While sensitive and specific, the procedure is invasive, costly, has limited availability and includes certain risks such as induction of infection and perforation of the bowel.

A commonly used and less expensive way of screening for CRC is a fecal occult blood test (FOBT), which tests for the presence of blood in faeces. The presence of haemoglobin as a representative blood protein in faeces is an indicator of intestinal bleeding, which is frequently associated with CRC. However, since occult in a fecal sample could be indicative of a variety of gastrointestinal disorders, further medical testing such as colonoscopy remains necessary to identify colorectal cancer.

Fecal occult blood tests fall primarily into two categories, tests based on the use of chromogenic chemical reagents such as gum guaiac and immunochemical tests. The chemically based guaiac methods determine the presence of occult blood by the detection of the perioxidase activity of the hemoglobin in the blood present in the faecal sample. They require catalysis of peroxide into oxygen and water, and the subsequent oxidation of a

colorless dye (most often into a colored form). However, peroxidase activity is also found in meats and vegetables. In order to produce accurate results, these tests require restriction of the intake of certain foods, drugs, vitamins, and other substances prior to and during the sample collection period. The sensitivity of the most commonly used guaiac FOBT (Hemoccult) is approximately 50%. Despite a specificity of 98%, the positive predictive value for FOBT is low. Methods of detecting occult blood based on porphyrin (heme and protpoporphyrin IX) analysis or immunologic tests using anti-hemoglobin antibodies improve on these results. Immunochemical tests (FIT or iFOBT) that use anti-hemoglobin antibodies specific for human blood in extracts from stool do not require dietary restrictions; however, they are more complicated and more expensive than peroxidase-based tests. In addition, human hemoglobin in fecal samples degrades with time, resulting in a loss of antigenicity which can produce false negative results. Reported sensitivity of these immunologic tests varies widely but is typically 60-80% depending on the population tested. Specificity is estimated to be -98%. Because of the intermittent nature of colorectal bleeding, the sensitivity of FOBT and FIT is directly proportional to the number of samples taken and the frequency of testing.

Recent developments in testing look specifically for mutations in DNA characteristic of colorectal neoplasia that are detectable in exfoliated epithelial cells in the stool (Pignone, et al., 2002; Ahlquist, et al., 2002). While neoplastic bleeding is intermittent, epithelial shedding is continual, potentially making stool-based DNA testing (i.e., also known as fecal DNA [f-DNA] and stool DNA [sDNA]) testing more sensitive than other methods. Early studies of molecular feacal screening primarily focused on single mutations. Gene mutations in P53, K-ras, and BAT 26, for instance, have been linked to colorectal cancer and remain detectable in feacal samples. Colorectal neoplasms are varied in nature and no single mutation has been identified as being expressed universally. For this reason, multiple target assay panels (MTAP) are preferably used. PreGen-Plus™ (EXACT Sciences Corporation, Maynard, MA; Laboratory Corporation of America, Burlington, NC) is a single test that identifies the presence of 23 different microsatellite (MSI) mutations known to be associated with CRC, including mutations in BAT-26. Additionally, 21 other point mutations in other genes associated with CRC are included in this test: APC, K-ras, and p53. This test is further

designed to detect long DNA fragments, which have been specifically associated with cells called non-apoptotic colonocytes, which are common in CRC. While this test is more sensitive than fecal occult blood testing, it is not as sensitive as colonoscopy and will miss about half of cancers in an average risk group of people without symptoms.

Increased DNA methylation is an epigenetic alteration that is common in human cancers and is often associated with transcriptional silencing. Aberrantly methylated DNA has also been proposed as a potential tumor marker for CRC detection. Genes such as vimentin, which are transcriptionally silent in normal epithelium, have been considered as targets for cancer-associated aberrant methylation and for use as cancer markers (JNCI Journal of the National Cancer Institute 2005 97(15):1124-1132). A combined assay utilizing hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY), demonstrated a sensitivity of 88% for CRC with a specificity of 82% (Am J Gastroenterol. 2008 Nov; 103(11):2862-70). Further, ColoSure® is a single marker laboratory developed, stool based DNA test. This method examines DNA in exfoliated colon cells for cancer-associated aberrant methylation of the vimentin gene and reaches a sensitivity range of 72-77% and a specificity range of 83-94% in average risk individuals.

Protein tests provide an alternative method for detecting CRC. Tests assessing the presence of tumor-derived enzymes such as M2 pyruvate kinase (M2-PK), and/or proteins such as calprotectin, carcinoembryonic antigen (CEA), tissue inhibitor of metalloproteinase-1 (TIMP-1) and S100 calcium binding protein A12 (S100A12) have been described. A diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers S100A12 and TIMP-1 has been described in Clin Gastroenterol Hepatol. 2008 Oct;6(10):1122-8. Dimeric isoenzyme of pyruvate kinase, M2-PK, expressed by tumor cells, has as well been proposed as a screening tool for CRC. The performance of fecal M2-PK has been evaluated with IFOBT and colonoscopy in Am J Gastroenterol. 2008 Jun;103(6):1496-504. Compared to immunochemical FOBTs, TuM2-PK does not have supplemental value for screening for CRC because of a lower sensitivity and specificity (Eur J Gastroenterol Hepatol. 2007 Oct;19(10):878-82)

Although combined assays for detecting CRC have been described, their approach targets either multiple protein markers or either multiple DNA alterations. To date, immunochemical tests and DNA tests for CRC detection have been evaluated and compared on a separate basis only.

EP0308227 describes a chemical fecal occult blood test employing a guaiac matrix.

EP0032782 describes a method for the detection of haemoglobin or decomposition products of haemoglobin in feces by means of an immunological reaction by using an antibody specific for human haemoglobin.

US7288413 describes methods that combine a chemical fecal occult blood test and an immunochemical fecal occult blood test.

WO 04/092709 concerns a fecal blood test involving the dispersement of a dye in toilet water.

EP0817968 describes several suitable stool collecting and testing methods and devices.

WO 05/017207 discloses that the vimentin gene can be a common target for methylation and epigenetic gene silencing in colon neoplasia, and may function as a candidate tumor suppressor gene.

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

WO 2006/113671 and WO 2008/010975 describe methylation markers relevant to colorectal cancer.

SUMMARY OF THE INVENTION

The invention provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising:

(a) detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer,

(b) detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer

and based upon a positive result obtained in either (a) or (b) or in both (a) and (b) detecting a predisposition to, or the incidence of, colorectal cancer.

Also described herein is a method of sample processing, prior to carrying out a method of the invention, comprising removing a portion of a collected faecal sample and adding the removed portion of the sample to a buffer which prevents denaturation or degradation of blood proteins found in the sample.

The invention also provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a sample comprising detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 , wherein detection of the epigenetic modification in at least one of the genes in the panel is indicative of a predisposition to, or the incidence of, colorectal cancer.

The invention also provides a method of detecting a predisposition to, or the incidence of, cancer (and in particular colorectal cancer) in a sample comprising detecting an epigenetic modification in at least one gene selected from LAMA1 and CDO1, wherein detection of the epigenetic modification in the at least one gene is indicative of a predisposition to, or the incidence of, cancer (and in particular colorectal cancer).

The invention also relates to a method of detecting a predisposition to, or the incidence of, colorectal cancer (in particular in a faecal sample) comprising detecting an epigenetic modification in at least one gene selected from GPNMB and MMP2, wherein detection of the epigenetic modification in the at least one gene is indicative of a predisposition to, or the incidence of, colorectal cancer.

In related aspects, the invention provides

a method for predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic modification in:

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene is indicative that the likelihood of successful treatment is higher than if the epigenetic modification is not detected.

a method for predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethyiating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic modification in

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 ,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene is indicative that the likelihood of resistance to treatment is lower than if the epigenetic modification is not detected.

a method of selecting a suitable treatment regimen for colorectal cancer comprising detecting an epigenetic modification in

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 ,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene results in selection of a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or a HDAC inhibitor for treatment and wherein if the epigenetic modification is not detected, a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or a HDAC inhibitor is not selected for treatment.

a method for monitoring treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic modification in

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE 1 ,

(b) at least one gene selected from LAMA1 and CDO1 ; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of a reduction in the epigenetic modification in at least one of the genes in the panel or in the at least one gene as treatment progresses is indicative of successful treatment.

Thus, the epigenetic modification may be measured at the start of the treatment and then once or more following treatment, or as treatment progresses, in order to determine if the treatment is achieving the desired effect. A return to lower levels of methylation of the genes is considered indicative of effective treatment.

The invention also relates to a kit for detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising:

(a) means for detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer, and

(b) means for detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer.

Also provided is a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) monitoring treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(d) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or

(e) selecting a suitable treatment regimen for colorectal cancer comprising means for detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1.

Similarly, the invention also provides a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or

(d) selecting a suitable treatment regimen for colorectal cancer comprising means for detecting an epigenetic modification in at least one gene selected from LAM A1 and CD01.

The invention also provides a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or (d) selecting a suitable treatment regimen for colorectal cancer comprising means for detecting an epigenetic modification in at least one gene selected from GPNMB and MMP2 and means for processing a faecal sample.

The invention also provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer, characterised in that the faecal sample has previously been stored for at least approximately 6 months, 1 , 2, 3, 4, 5, 6 or more years and/or is less than approximately 4, 3, 2, or 1g in weight.

DETAILED DESCRIPTION OF THE INVENTION

The invention, as set out in the claims, is based upon successful attempts to improve the detection of colorectal cancer. In particular, the invention aims to improve the positive and negative predictive value and also the sensitivity and specificity of detection of colorectal cancer through non-invasive means. The methods of the invention may permit effective detection of colorectal cancer without the requirement for relatively expensive, highly invasive and painful procedures such as digital rectal examination, colonoscopy and sigmoidoscopy to be performed. The invention is based upon a combination of tests for detecting proteins and epigenetic modification markers respectively in the same faecal sample, shown for the first time herein to provide a particularly useful overall test.

Thus, according to a first aspect, the invention provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising, consisting essentially of or consisting of:

(a) detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer,

(b) detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer

and based upon a positive result obtained in either (a) or (b) or in both (a) and (b) detecting a predisposition to, or the incidence of, colorectal cancer.

As shown herein, the combination of methylation marker assay and fecal occult blood test (FOBT) gives very specific and sensitive results.

The combined methods of the invention improve the negative predictive value of the existing single tests. By improving sensitivity, the number of false negative results is decreased and this improves negative predictive value.

Step (a) of the methods involves detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer. Blood in the faeces is an indicator of intestinal bleeding, which is frequently associated with colorectal cancer. Thus, detection of blood in the faecal sample is considered a "positive" result in step (a). Any suitable method for detecting the presence of blood in the sample may be employed. Often, the methods of detecting blood will rely upon detecting a representative blood protein in the faecal sample. In certain embodiments, detecting the presence of blood in the faecal sample comprises, consists essentially of or consists of detection of haemoglobin in the faecal sample. Detection may be through any suitable means, and includes all variants of fecal occult blood tests. The test may be chromogenic or immunological in certain embodiments. The test may rely upon peroxidase activity of hemoglobin. Chromogenic tests are well known and commercially available and may rely upon chemical reagents such as gum guaiac. In specific embodiments, haemoglobin in the faecal sample is detected through immunochemical means. This may involve anti-hemoglobin antibodies in certain embodiments. The term "antibody" or "antibodies" herein refers to an antibody or antibodies, or a derivative thereof that retains

specific binding activity. By specific binding activity is meant the ability to specifically bind to hemoglobin. Thus, such a reagent does not bind, or does not bind to a significant degree, to unrelated proteins found in the faecal sample. Any antibody or derivative may be employed. Thus, the antibody may be a monoclonal or polyclonal antibody. The derivative of the antibody that retains specific binding activity may comprise, consist essentially of or consist of a humanized version of a non-human antibody, a heavy chain antibody, a single domain antibody, a nanobody, a Fab fragment or scFv etc. in certain embodiments. Numerous techniques are available for producing antibodies and their derivatized forms, as would be well known to one skilled in the art.

As mentioned above, the combination of techniques maximises sensitivity of detection, without significantly compromising specificity. Thus, the threshold detection concentrations for detection of blood/hemoglobin in step (a) may be those typically employed in fecal occult blood tests. Adding in the step (b) test improves overall sensitivity by picking up additional positive samples. For example, in some embodiments, the result in step (a) is considered positive if the concentration of hemoglobin detected is more than between (about) 50 to (about) 150 ng/ml. In more specific embodiments, the result in step (a) is considered positive if the concentration of hemoglobin detected is more than (about) 100 ng/ml.

However, in other embodiments, the methods of the invention may be employed to improve the sensitivity of the step (a) method, whilst preventing a resultant loss in specificity. By lowering the threshold concentration of blood to be detected in the faecal sample to give a positive result in step (a), the sensitivity of the step (a) method is increased. In order to retain specificity, the step (b) method is employed on those samples in which low levels, that is to say lower than the typically used threshold, of blood were detected in step (a). A positive result from the step (b) method is required to confirm the positive result in step (a) for the "low level" samples. For those samples having blood (especially hemaglobin) concentrations above the typically employed threshold in step (a), it is not necessary to perform the method of step (b), since for these samples the step (a) method is sufficiently specific for this not to be necessary. This has the advantage that the step (b) test is not required for all samples, thus reducing costs and increasing throughput. Thus, in certain

embodiments, the result in step (a) is considered positive if the concentration of hemoglobin detected is lower than is typically employed as the threshold concentration of hemoglobin in hemoglobin detection tests, but for those samples in which a "lower than typical threshold" concentration of hemoglobin is detected, step (b) is performed on these samples. The detection of the epigenetic modification in step (b) is then used to confirm the positive result in step (a). The step (b) test is not employed for those samples in which the concentration of hemoglobin detected is higher than the threshold typically employed in hemoglobin detection tests.

In specific embodiments, the result in step (a) is considered positive if the concentration of hemoglobin detected is more than or at least (about) 5 to (about) 50 ng/ml, more specifically more than or at least (about) 5 to (about) 20 ng/ml and more particularly more than or at least (about) 10 ng/ml. By lowering the threshold, the sensitivity of the test is increased. In such embodiments, step (b) is performed only in the event that the concentration of hemoglobin detected is between (about) 5 ng/ml and (about) 250 ng/ml, more specifically between (about) 10 ng/ml and (about) 200 ng/ml. The detection of the epigenetic modification in step (b) is then used to confirm the positive result in step (a). Thus, a positive result in step (b) confirms the result in step (a) as positive. If no epigenetic modification of the DNA is detected, the result of step (a) is considered negative. For samples in which the concentration of hemoglobin detected is more than or at least (about) 200 ng/ml (or (about) 250 ng/ml), it is not necessary to perform step (b), since the result in step (a) will be of high specificity (i.e. is unlikely to be a false positive).

Step (b) involves detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer. Thus, detection of the epigenetic modification is considered a "positive" result in step (b).

In some embodiments, the epigenetic modification is detected in at least one gene selected from PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1 , LAMA1 , MMP2, OSMR, SFRP2 and CDO1, with detection of the epigenetic modification

in at least one of the genes providing an indication of a predisposition to, or incidence of, colorectal cancer.

In certain embodiments, the epigenetic modification is detected in at least one gene selected from PHACTR3, NDRG4 and FOXE1 , with detection of the epigenetic modification in at least one of the genes providing an indication of a predisposition to, or incidence of, colorectal cancer.

PHACTR3 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 20 (location 20q13.32-q13.33) and the gene sequence is listed under the accession numbers AJ311122 and NM_080672. The gene encodes the phosphatase and actin regulator 3.

NDRG4 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 16 (location q21-q22.3) and the gene sequence is listed under the accession number AB044947. The gene encodes NDRG family member 4.

FOXE1 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 9 (location 9q22) and the gene sequence is listed under the accession number U89995. The gene encodes the forkhead box E1 (thyroid transcription factor 2).

GATA4 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 8 (location 8p23.1-p22) and the gene sequence is listed under the accession numbers AK097060 and NMJ302052. The gene encodes the GATA binding protein 4.

GPNMB is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 7 (location 7p) and the gene sequence is listed under the accession numbers X76534 and NMJ301005340. The gene encodes the glycoprotein (transmembrane) nmb.

TFPI2 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 7 (location 7q) and the gene sequence is listed under the accession numbers L27624 and NM_006528. The gene encodes tissue factor pathway inhibitor 2.

SOX17 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 8 (location 8q11.23) and the gene sequence is listed under the accession number AB073988. The gene encodes SRY (sex determining region Y)-box 17.

SYNE1 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 6 (location 6q24.2-q25.3) and the gene sequence is listed under the accession number AB018339. The gene encodes spectrin repeat containing, nuclear envelope 1.

LAMA1 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 18 (location 18p11.3) and the gene sequence is listed under the accession numbers X58531 and NM_005559. The gene encodes laminin, alpha 1.

MMP2 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 16 (location 16q13-q21) and the gene sequence is listed under the accession number NM_001127891. The gene encodes matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase).

OSMR is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 5 (location 5p13.2) and the gene sequence is listed under the accession number U60805 and NM_003999. The gene encodes the oncostatin M receptor.

SFRP2 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 4 (location 4q31.3) and the gene sequence is listed under the accession number AF017986. The gene encodes the secreted frizzled-related protein 2.

CDO1 is the gene symbol approved by the HUGO Gene Nomenclature Committee. The gene is located on chromosome 5 (location 5q23.2) and the gene sequence is listed under the accession number NM_001801. The gene encodes the cysteine dioxygenase, type I.

By "gene" is meant the specific known gene in question. It may also relate to any gene which is taken from the family to which the named "gene" belongs, in certain circumstances, and includes according to all aspects of the invention not only the particular sequences found in the publicly available database entries, but also encompasses transcript and nucleotide variants of these sequences, with the proviso that methylation or another epigenetic modification of the gene is linked to the incidence of colorectal cancer. Variant sequences may have at least 90%, at least 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleotide sequence identity with the nucleotide sequences in the database entries. Computer programs for determining percentage nucleotide sequence identity are available in the art, including the Basic Local Alignment Search Tool (BLAST) available from the National Center for Biotechnology Information.

The methods of the invention are generally in vitro methods carried out on an isolated test sample. The methods are used to diagnose colorectal cancer. In certain embodiments the methods may also include the step of obtaining the sample. The test sample is generally from a human subject. The subject may be suspected of being tumorigenic. More specifically the subject may be suspected of suffering from a colorectal cancer.

In specific embodiments of the methods, the epigenetic modification is detected in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 , with detection of the epigenetic modification in at least one of the genes providing an indication of a predisposition to, or incidence of, colorectal cancer. Specific panels which may be employed comprise, consist essentially of or consist of PHACTR3, NDRG4 and FOXE1, NDRG4 and FOXE1 , PHACTR3 and NDRG4, or PHACTR3 and FOXE1.

In other embodiments, the epigenetic modification is detected in at least one gene selected from LAMA1 and CDO1. In further embodiments, the epigenetic modification is

detected in at least one gene selected from GPNMB and MMP2 The methods of the invention may have diagnostic and prognostic value, and this is included within the definition of the term "detecting a predisposition to, or the incidence of, colorectal cancer". The prognostic value of the methods of the invention may be used as a marker of potential susceptibility to colorectal cancer or as a marker for progression from adenoma to cancer for example. Thus patients at risk may be identified before the disease has a chance to manifest itself in terms of symptoms identifiable in the patient. Initial detection as well as follow-up detection, for example following treatment, is also included within the definition. Follow up detection may be performed after any treatment. Examples include following polypectomy (removal of polyps) and following surgery to remove a colorectal carcinoma, severe neoplasia or advanced adenoma.

The methods of the invention may be carried out on purified or unpurified DNA-containing samples. However, in specific embodiments, DNA is isolated/extracted/purified from the sample. Any suitable DNA isolation technique may be utilised. Examples of purification techniques may be found in standard texts such as Molecular Cloning - A Laboratory Manual (Third Edition), Sambrook and Russell (see in particular Appendix 8 and Chapter 5 therein). In some embodiments, purification involves alcohol precipitation of DNA. Preferred alcohols include ethanol and isopropanol. Suitable purification techniques also include salt-based precipitation methods. Thus, in specific embodiments the DNA purification technique comprises use of a high concentration of salt to precipitate contaminants. The salt may comprise, consist essentially of or consist of potassium acetate and/or ammonium acetate for example. The method may further include steps of removal of contaminants which have been precipitated, followed by recovery of DNA through alcohol precipitation.

In alternative embodiments, the DNA purification technique is based upon use of organic solvents to extract contaminants from cell lysates. Thus, in certain embodiments, the method comprises use of phenol, chloroform and isoamyl alcohol to extract the DNA. Suitable conditions are employed to ensure that the contaminants are separated into the organic phase and that DNA remains in the aqueous phase.

In specific embodiments of these purification techniques, extracted DNA is recovered through alcohol precipitation, such as ethanol or isopropanol precipitation.

Amplification of DNA (using PCR) from natural sources is often inhibited by co-purified contaminants and various methods adopted for DNA extraction from environmental samples are available and provide an alternative for isolating DNA from faecal samples, according to specific embodiments of the invention. For instance, the QIAamp DNA Stool Mini Kit from QIAGEN adsorbs DNA-damaging substances and PCR inhibitors present in the sample by InhibitEX. Other examples for application to faecal samples include the Wizard Genomic DNA Purification Kit (Promega), the NucliSENS® easyMAG™ (Biomerieux) and nucleic acid purification kits manufactured by Macherey Nagel.

The methods of the invention may also, as appropriate, incorporate quantification of isolated/extracted/purified DNA in the sample. Quantification of the DNA in the sample may be achieved using any suitable means. Quantitation of nucleic acids may, for example, be based upon use of a spectrophotometer, a fluorometer or a UV transilluminator. Examples of suitable techniques are described in standard texts such as Molecular Cloning - A Laboratory Manual (Third Edition), Sambrook and Russell (see in particular Appendix 8 therein). In some embodiments, kits such as the Picogreen® dsDNA quantitation kit available from Molecular Probes, Invitrogen may be employed to quantify the DNA.

The inventor has determined that faecal samples for use in the invention do not necessarily have to be freshly collected. This applies in particular for performing step (b) and thus also the further methods of the invention discussed herein where epigenetic modifications in specific genes are determined in faecal samples, Thus, samples collected 1, 2, 3, 4, 5, 6 or more years ago may be employed. The historical samples may have been frozen for storage. Freezing is performed at a suitable temperature, such as 20°C for example. Lyophilisation may be performed in certain embodiments to permit storage of the faecal samples. The sample may be frozen without the addition of a stabilizing buffer in certain embodiments as it has been shown that this is not necessary, when the sample is frozen, in order to retain DNA integrity in the sample. In other embodiments, a suitable

stabilizing buffer as known to those in the art, may be added to the sample before and/or after storage.

The inventor has also discovered that a minimal stool sample, including a minimal sample derived from a historical sample as discussed herein, may successfully be employed in the methods of the invention. This is particularly applicable to performing step (b) and thus is also applicable to the further aspects of the invention discussed herein where epigenetic modifications in specific genes are determined, in faecal samples. Thus, only a portion of a collected faecal sample need be employed in the methods of the invention in order to achieve reliable results. Reliable results may be achieved by isolating, extracting or purifying DNA from less than 5g of faecal sample, in particular from approximately 4, 3, 2, or 1g of faecal sample. The faecal sample may be present in an appropriate volume of homogenizing buffer to facilitate DNA isolation, extraction and purification.

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

Thus, the invention provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising, consisting essentially of or consisting of detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer, characterised in that the faecal sample has previously been stored for at least approximately 6 months, 1 , 2, 3, 4, 5, 6 or more years and/or is less than approximately 4, 3, 2, or 1g in weight (prior to addition of homogenization buffer).

The detailed discussion of determination of epigenetic modifications provided herein applies mutatis mutandis to this aspect of the invention.

"Colorectal cancer", also called colon cancer or bowel cancer, is defined to include cancerous growths, carcinomas, severe neoplasias and advanced adenomas in the colon, rectum and appendix.

"Epigenetic modification" is defined herein to include any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene. Epigenetic modifications may, in certain circumstances be stable heritable traits. A number of related mechanisms that cause alteration in gene expression are recognised and include DNA methylation, histone changes (for example changes in histone acetylation) which may lead to chromatin remodelling and RNA interference. In many cases, hypermethylation of DNA incorrectly switches off critical genes allowing cancers to develop and progress. In specific embodiments, the epigenetic modification is methylation. In particular, aberrant methylation, which may be referred to as hypermethylation, of the gene or genes is detected. Typically, the methylation status is determined in suitable CpG islands which are often found in the promoter region of the gene(s). The term "methylation", "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine at one or a plurality of CpG dinucleotides within a DNA sequence. CpG dinucleotides are typically concentrated in the promoter regions and exons of human genes. CpG dinucleotides susceptible to methylation may be found in the promoter region, exons and introns of human genes. Promoter, exon and intron regions can all thus be assessed for methylation. A "promoter" is a region extending typically between approximately 1 Kb, 500 bp or 150 to 300 bp upstream from the transcription start site. In some embodiments, the CpG island surrounding or positioned around the transcription start site is analysed to determine its methylation status. Alternatively, the methylation status of the exon and/or intron regions of may be determined as appropriate. The identification of CpG islands, to assess for determination of methylation status, is a matter of routine for one skilled in the art and various techniques, including in silico techniques, are available.

Since hypermethylation of a gene may result in diminished gene expression it may be possible, in certain embodiments, to carry out the methods of the invention by determining gene expression of the DNA in the faecal sample, rather than investigating the methylation status of the DNA directly. Many suitable techniques are available for determining gene expression at either the RNA or protein level. In certain embodiments, expression at the RNA level is determined by reverse transcriptase polymerase chain reaction (RT-PCR). In alternative embodiments, expression is determined at the protein level. Again, any suitable technique may be employed such as western blotting, ELISA etc.

Measurement of expression of a gene on its own may not necessarily conclusively indicate that the silencing is epigenetic, as the mechanism of silencing could be genetic, for example, by somatic mutation. Accordingly, in certain embodiments, the methods of the invention incorporate an appropriate re-expression assay which is designed to reverse epigenetic silencing. Appropriate treatment of the sample using a demethylating agent, such as a DNA-methyltransferase (DMT) inhibitor may reverse epigenetic silencing of the relevant gene. Suitable reagents include, but are not limited to, DAC (5'-deazacytidine), TSA or any other treatment affecting epigenetic mechanisms present in cell lines. Suitable reagents are discussed herein with respect to the pharmacogenetic, treatment monitoring and treatment aspects of invention, which discussion applies mutatis mutandis. Typically, expression is reactivated or reversed upon treatment with such reagents, indicating that the silencing is epigenetic.

Determination of the methylation status may be achieved through any suitable means. Suitable examples include bisulphite genomic sequencing and/or by methylation specific PCR. Various techniques for assessing methylation status are known in the art and can be used in conjunction with the present invention: sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR(McMS-PCR), MLPA with or without bisulphite treatment, QAMA (Zeschnigk et al, 2004), MSRE-PCR (Melnikov et al, 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulphite conversion-specific methylation-specific PCR (BS-MSP)(Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence diferences in PCR products of sodium bisulphite - treated DNA), methylation-sensitive single-nucleotide primer

extension conformation(MS-SNuPE), methylation- sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulphite restriction analysis (McCOBRA)(Akey et al., 2002), PyroMethA, HeavyMethyl (Cottrell et al. 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional methylation assay (ERMA)1 QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques for DNA methylation analysis is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264, Nature Reviews, 2003, Vol.3, 253-266; Oral Oncology, 2006, Vol. 42, 5-13, which references are incorporated herein in their entirety.

Techniques for assessing methylation status are based on distinct approaches. Some include use of endonucleases. Such endonucleases may either preferentially cleave methylated recognition sites relative to non-methylated recognition sites or preferentially cleave non-methylated relative to methylated recognition sites. Some examples of the former are Ace III, Ban I, BstN I, Msp I, and Xma I. Examples of the latter are Ace II, Ava I, BssH II, BstU I, Hpa II, and Not I. Differences in cleavage pattern are indicative for the presence or absence of a methylated CpG dinucleotide. Cleavage patterns can be detected directly, or after a further reaction which creates products which are easily distinguishable. Means which detect altered size and/or charge can be used to detect modified products, including but not limited to electrophoresis, chromatography, and mass spectrometry.

Alternatively, the identification of methylated CpG dinucleotides may utilize the ability of the methyl binding domain (MBD) of the MeCP2 protein to selectively bind to methylated DNA sequences (Cross et al, 1994; Shiraishi et al, 1999). The MBD may also be obtained from MBP, MBP2, MBP4, poly-MBD (Jorgensen et al., 2006) or from reagents such as antibodies binding to methylated nucleic acid. The MBD may be immobilized to a solid matrix and used for preparative column chromatography to isolate highly methylated DNA sequences. Variant forms such as expressed His-tagged methyl-CpG binding domain may be used to selectively bind to methylated DNA sequences. Eventually, restriction endonuclease digested genomic DNA is contacted with expressed His-tagged methyl-CpG binding domain. Other methods are well known in the art and include amongst others methylated-CpG island recovery assay (MIRA). Another method, MB- PCR, uses a

recombinant, bivalent methyl-CpG-binding polypeptide immobilized on the walls of a PCR vessel to capture methylated DNA and the subsequent detection of bound methylated DNA by PCR.

Further approaches for detecting methylated CpG dinucleotide motifs use chemical reagents that selectively modify either the methylated or non-methylated form of CpG dinucleotide motifs. Suitable chemical reagents include hydrazine and bisulphite ions. The methods of the invention preferably use bisulphite ions. The bisulphite conversion relies on treatment of DNA samples with sodium bisulphite which converts un methylated cytosine to uracil, while methylated cytosines are maintained (Furuichi et al., 1970). This conversion finally results in a change in the sequence of the original DNA. It is general knowledge that the resulting uracil has the base pairing behaviour of thymidine which differs from cytosine base pairing behaviour. This makes the discrimination between methylated and non-methylated cytosines possible. Useful conventional techniques of molecular biology and nucleic acid chemistry for assessing sequence differences are well known in the art and explained in the literature. See, for example, Sambrook, J., et al., Molecular cloning: A laboratory Manual, (2001) 3rd edition, Cold Spring Harbor, NY; Gait, M.J.(ed.), Oligonucleotide Synthesis, A Practical Approach, IRL Press (1984); Hames B.D., and Higgins, SJ. (eds.), Nucleic Acid Hybridization, A Practical Approach, IRL Press (1985); and the series, Methods in Enzymology, Academic Press, Inc.

Some techniques use primers for assessing the methylation status at CpG dinucleotides. Two approaches to primer design are possible. Firstly, primers may be designed that themselves do not cover any potential sites of DNA methylation. Sequence variations at sites of differential methylation are located between the two primers and visualisation of the sequence variation requires further assay steps. Such primers are used in bisulphite genomic sequencing, COBRA, Ms-SnuPE and several other techniques. Secondly, primers may be designed that hybridize specifically with either the methylated or unmethylated version of the initial treated sequence. After hybridization, an amplification reaction can be performed and amplification products assayed using any detection system known in the art. The presence of an amplification product indicates that a sample hybridized to the primer. The specificity of the primer indicates whether the DNA had been modified or

not, which in turn indicates whether the DNA had been methylated or not. If there is a sufficient region of complementarity, e.g., 12, 15, 18, or 20 nucleotides, to the target, then the primer may also contain additional nucleotide residues that do not interfere with hybridization but may be useful for other manipulations. Examples of such other residues may be sites for restriction endonuclease cleavage, for ligand binding or for factor binding or linkers or repeats. The oligonucleotide primers may or may not be such that they are specific for modified methylated residues.

A further way to distinguish between modified and unmodified nucleic acid is to use oligonucleotide probes. Such probes may hybridize directly to modified nucleic acid or to further products of modified nucleic acid, such as products obtained by amplification. Probe-based assays exploit the oligonucleotide hybridisation to specific sequences and subsequent detection of the hybrid. There may also be further purification steps before the amplification product is detected e.g. a precipitation step. Oligonucleotide probes may be labelled using any detection system known in the art. These include but are not limited to fluorescent moieties, radioisotope labelled moieties, bioluminescent moieties, luminescent moieties, chemiluminescent moieties, enzymes, substrates, receptors, or ligands.

In the MSP approach, DNA may be amplified using primer pairs designed to distinguish methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulphite treatment (Herman et al.,1996; and WO 97/46705). For example, bisulphite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. Thus an oligonucleotide primer which comprises adenine bases in place of guanine bases would hybridize to the bisulphite-modified DNA, whereas an oligonucleotide primer containing the guanine bases would hybridize to the non-modified (methylated) cytosine residues in the DNA. Amplification using a DNA polymerase and a second primer yield amplification products which can be readily observed, which in turn indicates whether the DNA had been methylated or not Whereas PCR is a preferred amplification method, variants on this basic technique such as nested PCR and multiplex PCR are also included within the scope of the invention.

As mentioned earlier, a preferred embodiment for assessing the methylation status of the relevant gene requires amplification to yield amplification products. The presence of amplification products may be assessed directly using methods well known in the art. They simply may be visualized on a suitable gel, such as an agarose or polyacrylamide gel. Detection may involve the binding of specific dyes, such as ethidium bromide, which intercalate into double-stranded DNA and visualisation of the DNA bands under a UV illuminator for example. Another means for detecting amplification products comprises hybridization with oligonucleotide probes. Alternatively, fluorescence or energy transfer can be measured to determine the presence of the methylated DNA.

A specific example of the MSP technique is designated real-time quantitative MSP (QMSP), and permits reliable quantification of methylated DNA in real time or at end point. Real-time methods are generally based on the continuous optical monitoring of an amplification procedure and utilise fluorescently labelled reagents whose incorporation in a product can be quantified and whose quantification is indicative of copy number of that sequence in the template. One such reagent is a fluorescent dye, called SYBR Green I that preferentially binds double-stranded DNA and whose fluorescence is greatly enhanced by binding of double-stranded DNA. Alternatively, labeled primers and/or labeled probes can be used for quantification. They represent a specific application of the well known and commercially available real-time amplification techniques such as TAQMAN®, MOLECULAR BEACONS®, AMPLIFLUOR® and SCORPION® DzyNA®, PlexorTM etc. In the real-time PCR systems, it is possible to monitor the PCR reaction during the exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template.

In specific embodiments, methylation specific PCR/amplification is utilised. This may be carried out in real time or at end point. The real time or end point PCR/amplification may involve use of hairpin primers (Amplifluor), hairpin probes (Molecular Beacons), hydrolytic probes (Taqman), FRET probe pairs (Lightcycler), primers incorporating a hairpin probe (Scorpion), fluorescent dyes (SYBR Green etc.), primers incorporating the complementary sequence of a DNAzyme and a cleavable fluorescent DNAzyme substrate or oligonucleotide blockers, for example.

Real-Time PCR detects the accumulation of amplicon during the reaction. Real-time methods do not need to be utilised, however. Many applications do not require quantification and Real-Time PCR is used only as a tool to obtain convenient results presentation and storage, and at the same time to avoid post-PCR handling. Thus, analyses can be performed only to confirm whether the target DNA is present in the sample or not. Such end-point verification is carried out after the amplification reaction has finished. This knowledge can be used in a medical diagnostic laboratory to detect a predisposition to, or the incidence of, cancer in a patient. End-point PCR fluorescence detection techniques can use the same approaches as widely used for Real Time PCR. For example, «Gene» detector allows the measurement of fluorescence directly in PCR tubes.

In real-time embodiments, quantitation may be on an absolute basis, or may be relative to a constitutively methylated DNA standard, or may be relative to an unmethylated DNA standard. Methylation status may be determined by using the ratio between the signal of the marker under investigation and the signal of a reference gene where methylation status is known (such as β-actin for example), or by using the ratio between the methylated marker and the sum of the methylated and the non-methylated marker. Alternatively, absolute copy number of the methylated marker gene can be determined.

Suitable controls may need to be incorporated in order to ensure the method chosen is working correctly and reliably. Suitable controls may include assessing the methylation status of a gene known to be methylated. This experiment acts as a positive control to ensure that false negative results are not obtained. The gene may be one which is known to be methylated in the sample under investigation or it may have been artificially methylated, for example by using a suitable methyltransferase enzyme, such as Sssl methyltransferase.

Additionally or alternatively, suitable negative controls may be employed with the methods of the invention. Here, suitable controls may include assessing the methylation status of a gene known to be unmethylated or a gene that has been artificially demethylated. This experiment acts as a negative control to ensure that false positive results are not obtained.

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

In specific embodiments, the methods utilise primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth in table 1. Suitable primer pairs can be selected from the primers listed in the table based upon the gene or genes of interest. In specific embodiments, the methods utilise primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 1 and 2 (PHACTR3), 4 and 5 (FOXE1), and 7 and 8 (NDRG4) in order to detect methylation status in the DNA. These primers are particularly useful in MSP based methods of detection. Each of the primers covers one or more methylation sites and is specific for the methylated sequence following bisulphite treatment.

In certain embodiments, the methods utilise probes selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth in table 1. Suitable probes can be selected from the probes listed in the table based upon the gene or genes of interest. In specific embodiments, the methods utilise probes selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 3 (PHACTR3), 6 (FOXE1), and 9 (NDRG4) in order to detect methylation status in the DNA. Each of the probes covers one or more methylation sites and is specific for the methylated sequence following bisulphite treatment. The probes may be utilised in

combination with the primers listed above in specific embodiments. The probes may be fluorescently labelled, in particular with a donor and an acceptor (or quencher) fluorophore to permit FRET to occur and allow real-time or end-point detection. An example of a donor moiety is FAM and an example of an acceptor or quencher moiety is DABCYL.

The methods of the invention are performed using a faecal sample. In certain embodiments, a single faecal sample is utilised as the source of the sample for (a) and (b). However, the sample may need to be split to permit (a) and (b) to be carried out. Thus the sample may need to be treated to differing degrees in order to permit the separate steps to be carried out. As a result, after obtaining the faecal sample, the sample may be apportioned appropriately to allow the DNA and protein testing to be carried out separately.

In the context of these methods of the invention, there is the requirement for appropriate sample processing, since two separate detection techniques may be performed on a single sample. To meet this requirement, the invention provides a method of sample processing, prior to carrying out a method of the invention (tests (a) and (b)), comprising removing a portion of a collected faecal sample and adding the removed portion of the sample to a buffer which prevents denaturation or degradation of blood proteins found in the sample. The faecal sample may be collected at home by the subject. This may involve defecation into a suitable vessel. From this sample is removed a sufficient portion of the sample. This removed portion is then added to a suitable container, which may already contain a buffer. Alternatively, the buffer may be added with the removed portion of the sample or (shortly) afterwards. The removed portion is thus prepared for performance of step (a), namely detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer, on this sample. The buffer prevents denaturation or degradation of blood proteins found in the sample. Suitable buffers for protein preservation are known in the art and commercially available. The remaining portion of the sample may remain in the vessel in which it was originally collected, prior to carrying out step (b), namely detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer. Suitable buffers for maintaining DNA integrity may be added to the remaining

portion of the sample, in certain embodiments. The separated samples may be suitably labelled to ensure that they are paired up correctly during subsequent processing, including data analysis and comparison.

Following collection of the sample and suitable processing, the method may further comprise forwarding or otherwise delivering the removed portion of the collected faecal sample to a laboratory for performing step (a) of the method, as defined herein, on the removed portion of the collected faecal sample. The forwarding or otherwise delivering may occur within any suitable time frame following sample collection. In certain embodiments, the forwarding or otherwise delivering may occur within 6, 12, 24, 48 or 72 hours of sample collection. The method may then further comprise performing step (a) of the method on the removed portion of the collected faecal sample once it has been delivered. As described herein, step (b) may only be performed on a specific subset of samples in which blood is detected at low levels. Thus, the methods of sample processing may involve sorting those samples with detection of low levels of blood detected in step (a) (as defined herein) for testing in step (b). The appropriate remaining portions of the collected samples in which step (b) is not required (i.e. where a confirmation of the result in step (a) is not required - either because the no blood is detected or the level of blood is above the typically employed threshold (such as 200 ng/ml)) may then be discarded as appropriate.

Similarly, following collection of the sample and suitable processing, the method may further comprise forwarding or otherwise delivering the remaining portion of the collected faecal sample to a laboratory for performing step (b) of the method, as defined herein, on the remaining portion of the collected faecal sample. The forwarding or otherwise delivering may occur within any suitable time frame following sample collection. In certain embodiments, the forwarding or otherwise delivering may occur within 6, 12, 24, 48 or 72 hours of sample collection. The method may then further comprise performing step (b) of the method on the remaining portion of the collected faecal sample once it has been delivered.

In certain embodiments, the forwarding or other delivery of the removed and remaining portions of the collected faecal sample may be carried out to the same location, optionally at the same time. This is the case where the same laboratory is carrying out both

steps (a) and (b) which may be the case where the laboratory has the capacity to perform both types of test.

As discussed herein, the faecal samples for use in the invention do not necessarily have to be freshly collected. The discussion above applies mutatis mutandis here. Similarly, a minimal stool sample, including a minimal sample derived from a historical sample as discussed herein, may successfully be employed in the methods of the invention. This is particularly applicable to performing step (b) (i.e. on the remaining portion of the faecal sample). Again, the discussion above applies mutatis mutandis here, including relating to pooling of samples during the DNA extraction process. The requirement for minimal or portioned samples for step (b) may be advantageous by permitting more of the sample to be utilised to perform step (a), potentially improving the sensitivity of step (a).

As shown herein, methylation status determination based upon a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 can provide sensitive and specific detection of colorectal cancer. Thus, according to a related aspect, the invention provides a method of detecting a predisposition to, or the incidence of, colorectal cancer in a sample comprising detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1, wherein detection of the epigenetic modification in at least one of the genes in the panel is indicative of a predisposition to, or the incidence of, colorectal cancer.

It is also shown herein that methylation of LAMA1 and CDO1 is linked to the incidence of colorectal cancer. These genes have not been previously linked to colorectal cancer. Thus, the invention also provides a method of detecting a predisposition to, or the incidence of, cancer (and in particular colorectal cancer) in a sample comprising detecting an epigenetic modification in at least one gene selected from LAMA1 and CDO1 , wherein detection of the epigenetic modification in the at least one gene is indicative of a predisposition to, or the incidence of, cancer (and in particular colorectal cancer).

Evidence is also presented herein that methylation of GPNMB and MMP2 is linked to colorectal cancer, in particular based upon faecal samples. Thus, the invention also relates to

a method of detecting a predisposition to, or the incidence of, colorectal cancer (in particular in a faecal sample) comprising detecting an epigenetic modification in at least one gene selected from GPNMB and MMP2, wherein detection of the epigenetic modification in the at least one gene is indicative of a predisposition to, or the incidence of, colorectal cancer.

As for the first aspect of the invention, the epigenetic modification is often methylation and thus these methods may involve determining the methylation status of the at least one gene or panel of genes. Thus, aberrant methylation, or "hypermethylation", of the gene(s) may be detected. Various methods for determining methylation status are discussed herein, which discussion applies mutatis mutandis to these aspects of the invention. In specific embodiments, methylation specific PCR/amplification is utilised. This may be carried out in real time or at end point. The real time or end point PCR/amplification may involve use of hairpin primers (Amplifluor), hairpin probes (Molecular Beacons), hydrolytic probes (Taqman), FRET probe pairs (Lightcycler), primers incorporating a hairpin probe (Scorpion), fluorescent dyes (SYBR Green etc.), primers incorporating the complementary sequence of a DNAzyme and a cleavable fluorescent DNAzyme substrate or oligonucleotide blockers, for example. The methods may apply suitable primers and probes selected from table 1. In specific embodiments, the methods utilise primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 1 and 2 (PHACTR3), 4 and 5 (FOXE1), and 7 and 8 (NDRG4). In further embodiments, the methods utilise probes selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 3 (PHACTR3), 6 (FOXE1), and 9 (NDRG4). In other embodiments, the methods utilise primers selected from primers comprising the nucleotide sequences set forth as SEQ ID Nos: 28 and 29 (LAMA1) and 34 and 35 (CDO1). In further related embodiments, the methods utilise probes selected from probes comprising the nucleotide sequences set forth as SEQ ID Nos: 30 (LAMA1) and 36 (CDO1). In other embodiments, the methods utilise primers selected from primers comprising the nucleotide sequences set forth as SEQ ID Nos: 22 and 23 (GPNMB) and 31 and 32 (MMP2). In related embodiments, the methods utilise probes selected from probes comprising the nucleotide sequences set forth as SEQ ID Nos: 24 (GPNMB) and 33 (MMP2).

These methods of the invention may be ex vivo or in vitro methods carried out on a test sample. The methods may be non-invasive. The methods may be used to identify any stage of colorectal cancer, including pre-malignancies such as adenomas right through to carcinomas (see the definition of colorectal cancer herein).

The "sample" in which the epigenetic modification is detected may comprise, consist essentially of or consist of a tissue sample, a faecal sample or a blood sample. In preferred embodiments, the sample is a faecal sample. Historical samples (such as those collected at least approximately 1 year ago) and small samples (such as those of approximately 1g) may be useful in the methods of the invention, as discussed herein (which discussion applies mutatis mutandis). In specific embodiments, the tissue sample comprises, consists essentially of or consists of a colon and/or rectum and/or appendix sample. However, the sample may be from any representative tissue sample, body fluid, body fluid precipitate or lavage specimen, as required provided that detection of the epigenetic modification in the sample provides a reliable indicator of colorectal cancer. The sample may be obtained from a human subject. Test samples for diagnostic, prognostic, or personalised medicinal uses can be obtained from surgical samples, such as biopsies or fine needle aspirates, from paraffin embedded tissues, from frozen tumor tissue samples, from fresh tumor tissue samples, from a fresh or frozen body fluid, for example. Non-limiting examples include whole blood, bone marrow, cerebral spinal fluid, peritoneal fluid, pleural fluid, lymph fluid, serum, plasma, urine, chyle, stool, ejaculate, sputum, nipple aspirate, saliva, swabs specimen, wash or lavage fluid and/or brush specimens.

These methods may also include the step of obtaining the test sample, in certain embodiments. The tissue sample or liquid sample comprising the nucleic acid may be lysed or need to be concentrated to create a mixture of biological compounds comprising nucleic acids and other components. Alternatively, the nucleic acid may need to be cleared of proteins or other contaminants, e.g. by treatment with proteinase K. Procedures for lysing or concentrating biological samples are known by the person skilled in the art and can be chemical, enzymatic or physical in nature. A combination of these procedures may be applicable as well. For instance, lysis may be performed using ultrasound, high pressure, shear forces, alkali, detergents or chaotropic saline solutions, or proteases or lipases. For the

lysis procedure to obtain nucleic acids, or concentrating nucleic acid from samples, reference may be made to Sambrook, J., et al., Molecular cloning: A Laboratory Manual, (2001) 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York Ausubel, F.M., et al., Current Protocols in Molecular Biology (1987), J. Wiley and Sons, New York.

The test sample is generally obtained from a (human) subject suspected of being tumorigenic. Alternatively the test sample is obtained from a subject undergoing routine examination and not necessarily being suspected of having a disease. Thus patients at risk can be identified before the disease has a chance to manifest itself in terms of symptoms identifiable in the patient.

Alternatively the sample is obtained from a subject undergoing treatment, or from patients being checked for recurrence of disease.

Cancers characterised by hypermethylation of tumour suppressor genes may be treated by reducing methylation within the tumour cells. Various agents may be employed for this purpose, including DNA demethylating agents, DNA methyltransferase inhibitors and HDAC inhibitors. Accordingly, the invention also provides a method for predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic modification in: (a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 ,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene is indicative that the likelihood of successful treatment is higher than if the epigenetic modification is not detected.

Similarly, the invention provides a method for predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic

modification in (a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 ,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene is indicative that the likelihood of resistance to treatment is lower than if the epigenetic modification is not detected.

Furthermore, there is provided a method of selecting a suitable treatment regimen for colorectal cancer comprising detecting an epigenetic modification in (a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1, (b) at least one gene selected from LAMA1 and CDO1 ; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of the epigenetic modification in at least one of the genes in the panel or in the at least one gene results in selection of a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or a HDAC inhibitor for treatment and wherein if the epigenetic modification is not detected, a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or a HDAC inhibitor is not selected for treatment.

In a related aspect, the invention provides a method for monitoring treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor comprising detecting an epigenetic modification in

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1,

(b) at least one gene selected from LAMA1 and CDO1 ; or (c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) wherein detection of a reduction in the (levels of the) epigenetic modification in at least one of the genes in the panel or in the at least one gene as treatment progresses is indicative of successful treatment. Thus, the epigenetic modification may be measured at the start of the treatment and then once or more

following treatment, or as treatment progresses, in order to determine if the treatment is achieving the desired effect. A return to lower levels of methylation of the genes is considered indicative of effective treatment. Thus, the method may involve testing samples taken at various time points in order to determine the effect of the treatment. A threshold may be set (i.e. a level of reduction in the epigenetic modification) at which treatment is considered successful and may, therefore, be reduced or terminated.

For all of the relevant methods (pharmacogenetic methods, treatment regimen methods, monitoring methods and methods of treatment) of the invention, the DNA demethylating agent may be any agent capable of alleviating hypermethylation of the relevant genes (and thus up regulating transcription of the genes). The DNA methyltransferase inhibitor may be any suitable inhibitor of DNA methyltransferase activity or expression which is suitable for treating cancer in the presence of methylation of the at least one gene. The DNA methyltransferase inhibitor may, be one which reduces expression of DNMT genes, such as suitable antisense molecules, or double stranded RNA molecules, such as siRNA and miRNA molecules which mediate RNAi for example. The design of a suitable siRNA molecule is within the capability of the skilled person and suitable molecules can be made to order by commercial entities (see for example, www.ambion.com). In embodiments, the DNA methyltransferase gene is (human) DNMT1.

Alternatively, the agent may be a direct inhibitor of DNMTs. Examples include modified nucleotides such as phosphorothioate modified oligonucleotides (fig 6 of Villar-Garea, A. And Esteller, M. DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Current Drug Metabolism, 2003, 4, 11-31) and nucleosides and nucleotides such as cytidine analogues. Suitable examples of cytidine analogues include 5-azacytidine, 5-aza-2'-deoxycytidine, 5-fluouro-2'-deoxycytidine, pseudoisocytidine, 5,6-dihydro-5-azacytidine, 1-β-D- arabinofuranosyl-5-azacytosine (known as fazabarine) (see figure 4 of Villar-Garea, A. And Esteller, M. DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Current Drug Metabolism, 2003, 4, 11-31). The DNA methyltransferase inhibitor may comprise Decitabine.

Additional DNMT inhibitors include S-Adenosyl-Methionine (SAM) related compounds like ethyl group donors such as L-ethionine and non-alkylating agents such as S-adenosyl-homocysteine (SAH), sinefungin, (S)-6-methyl-6-deaminosine fungin, 6-deaminosinefungin, N4-adenosyl-N4-methyl-2,4-diaminobutanoic acid, 5'-methylthio-5'-deoxyadenosine (MTA)and 5'-amino-5'-deoxyadenosine (Villar-Garea, A. And Esteller, M. DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Current Drug Metabolism, 2003, 4, 11-31). Useful DNMT inhibitors in the present invention comprise, consists essentially of or consists of 5-azacytidine and/or zebulaine.

Further agents which may alter DNA methylation and which may, therefore, be useful in the present invention as DNA demethylating agents include organohalogenated compounds such as chloroform etc, procianamide, intercalating agents such as mitomycin C, 4-aminobiphenyl etc, inorganic salts of arsenic and selenium and antibiotics such as kanamycin, hygromycin and cefotaxim (Villar-Garea, A. And Esteller, M. DNA demethylating agents and chromatin-remodelling drugs: which, how and why? Current Drug Metabolism, 2003, 4, 11-31).

Many HDAC inhibitors are similarly known in the art. Examples include carboxylic acid based HDAC inhibitors such as valproate and/or butyrate and hydroxamic acid based HDAC inhibitors such as trichostatin A, suberoyl hydroxamic acid (SBHA), 6-(3-chlorophenylureido)caproic hydroxamic acid (3-CI-UCHA), m-carboxycinnamic acid bishydroxylamide (CBHA), suberoylanilide hydroxamic acid (SAHA), azelaic bishydroxamic acid (ABHA), pyroxamide, aromatic sulfonamides bearing a hydroxamic acid group and cyclic-hydroxamic-acid containing peptides.

For each of these additional aspects, the embodiments and optional features of the methods of the invention apply mutatis mutandis and are not repeated for reasons of conciseness. Thus, all methods of detecting an epigenetic modification in the DNA, in particular in the specified genes, including expression and re-expression based assays, may be employed appropriately.

In a further related aspect, the invention provides a method of treating colorectal cancer in a subject comprising administration of a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or a HDAC inhibitor wherein the subject has been selected for treatment on the basis of a method of the invention. Thus, detecting an epigenetic modification in

(a) a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 ,

(b) at least one gene selected from LAMA1 and CDO1; or

(c) at least one gene selected from GPNMB and MMP2 (in a faecal sample) may be used in order to direct treatment of the subject (from which the sample was taken).

The invention also relates to corresponding kits for carrying out the methods of the invention. Thus, the invention provides a kit for detecting a predisposition to, or the incidence of, colorectal cancer in a faecal sample comprising:

(a) means for detecting the presence of blood in the faecal sample, wherein detection of the presence of blood is indicative of a predisposition to, or the incidence of, colorectal cancer, and

(b) means for detecting an epigenetic modification in the DNA contained within the faecal sample, wherein detection of the epigenetic modification is indicative of a predisposition to, or the incidence of, colorectal cancer.

As for the corresponding methods, in certain embodiments, the epigenetic modification is detected in at least one gene selected from PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2 and CDO1 and thus the means for detecting an epigenetic modification in the DNA are means for detecting an epigenetic modification in at least one gene selected from PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2 and CDOl In further embodiments, the epigenetic modification is detected in at least one gene selected from PHACTR3, NDRG4 and FOXE1 and thus the means for detecting an epigenetic

modification in the DNA are means for detecting an epigenetic modification in at least one gene selected from PHACTR3, NDRG4 and FOXE1. The epigenetic modification may be detected in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 , with detection of the epigenetic modification in at least one of the genes providing an indication of a predisposition to, or incidence of, colorectal cancer. Means may be provided for detecting an epigenetic modification in a panel of genes comprising, consisting essentially of or consisting of PHACTR3, NDRG4 and FOXE1, NDRG4 and FOXE1, PHACTR3 and NDRG4, or PHACTR3 and FOXE1. In some embodiments, the means for detecting an epigenetic change in the panel of genes enable the detection to be carried out in a single reaction. Thus the means may permit multiplexing.

In further embodiments, the epigenetic modification is detected in at least one gene selected from LAMA1 and CDO1 and thus the means for detecting an epigenetic modification in the DNA are means for detecting an epigenetic modification in at least one gene selected from LAMA1 and CD01. Suitable primers and probes are set forth in table 1.

In further embodiments, the epigenetic modification is detected in at least one gene selected from GPNMB and MMP2 and thus the means for detecting an epigenetic modification in the DNA are means for detecting an epigenetic modification in at least one gene selected from GPNMB and MMP2. Suitable primers and probes are set forth in table 1.

As for the methods, the epigenetic modification is often methylation. Thus, the means for detecting an epigenetic modification in the DNA contained within the faecal sample may comprise, consist essentially of or consist of primers and/or probes which permit the methylation status of the DNA to be determined directly. In specific embodiments, the primers are selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth in table 1. Suitable primer pairs can be selected from the primers listed in the table based upon the gene or genes of interest. In specific embodiments, the kits incorporate primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as ~~as~~ SEQ ID Nos: 1 and 2 (PHACTR3), 4 and 5 (FOXE1), and 7 and 8 (NDRG4). In further specific embodiments, the probes are selected from probes comprising, consisting essentially of or consisting of the nucleotide

sequences set forth in table 1. Suitable probes can be selected from the probes listed in the table based upon the gene or genes of interest. In specific embodiments, the kits incorporate probes selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 3 (PHACTR3), 6 (FOXE1), and 9 (NDRG4).

The kit may contain any suitable means for allowing detection of blood in a faecal sample. In particular embodiments, the means for detecting the presence of blood in the faecal sample comprise means for detection of haemoglobin in the faecal sample. These may be chromogenic or immunochemical means. Thus, gum guaiac may be incorporated in the kits for chromogenic detection. Alternatively suitable anti-haemoglobin antibodies, as discussed herein, may be incorporated in the kits of the invention. These components may be provided together with suitable buffers etc. as would be readily appreciated by one skilled in the art.

The kits of the invention may also contain further elements to facilitate sample collection and processing. Thus in certain embodiments, the kit further comprises a sealable vessel for collection of a faecal sample. This vessel may be of a size and construction to permit a subject to directly defecate into the vessel. There may also be provided an additional container to which a removed portion of the sample may be added to allow step (a) to be performed on this separated sample. As mentioned in the methods section, this container may contain or be supplied with an appropriate buffer for protein preservation (to prevent denaturation or degradation of proteins). The kit may also contain suitable means for transfer of the removed portion of the sample to the second container. In view of the fact that the invention may be performed using only a small portion of the (total collected) faecal sample, as discussed herein (for example using as little as approximately 1 g of stool to perform DNA extraction), the second container may be suitably dimensioned to contain such a minimal/portioned sample. The second container may be provided with a suitable collection device, adapted to retrieve and/or deposit an appropriate minimal/portioned sample (as described herein, of approximately 5, 4, 3, 2 or 1g or less). Suitable tubes and spoons for inclusion in such a kit are known in the art and commercially available. The spoons may be measuring spoons and may be integrated into the tubes in certain embodiments. The container may be dimensioned such that a suitable volume of homogenization buffer (as

discussed herein, which discussion applies mutatis mutandis) may be added to the minimal/portioned sample added to the container.

In further embodiments, the kit, or more specifically the means for detecting an epigenetic modification in the DNA, further comprises means for processing a faecal sample. The means for processing a faecal sample comprises a homogenization buffer in certain embodiments. Suitable homogenization buffers are known in the art and commercially available. In related embodiments, the means for processing a faecal sample comprises reagents for extraction/isolation/concentration/purification of DNA. As an example, the QIAamp DNA stool kit available from Qiagen includes suitable components for purification of total DNA from fresh or frozen faecal samples.

In order to permit determination of the methylation status of the DNA, the kits may further comprise a reagent which selectively modifies unmethylated cytosine residues in the DNA contained in the sample to produce detectable modified residues but which does not modify methylated cytosine residues. As discussed above, this permits sequence differences to be detected as an indication of the methylation status of the DNA. Any suitable reagent may be employed. Many examples are known in the art and commercially available. In certain embodiments, the reagent comprises a bisulphite reagent. More specifically, the bisulphite reagent may comprise, consist essentially of or consist of sodium bisulphite.

In a related aspect, the invention also provides a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) monitoring treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(d) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or

(e) selecting a suitable treatment regimen for colorectal cancer comprising means for detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1.

As discussed herein, detection of the methylation status of this panel of genes provides a sensitive and specific means for detecting colorectal cancer. In certain embodiments, the means for detecting an epigenetic modification in a panel of at least two genes selected from PHACTR3, NDRG4 and FOXE1 comprise primers and/or probes which permit the methylation status of the genes to be determined directly. In specific embodiments, the primers are selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 1 and 2 (PHACTR3), 4 and 5 (FOXE1), and 7 and 8 (NDRG4). In further embodiments, the probes are selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 3 (PHACTR3), 6 (FOXE1), and 9 (NDRG4).

Similarly, the invention also provides a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or (d) selecting a suitable treatment regimen for colorectal cancer comprising means for detecting an epigenetic modification in at least one gene selected from LAMA1 and CDOl

As discussed herein, detection of the methylation status of these genes has been shown herein to provide a sensitive and specific means for detecting colorectal cancer. In

certain embodiments, the means for detecting an epigenetic modification in at least one gene selected from LAMA1 and CDO1 comprise primers and/or probes which permit the methylation status of the at least one gene to be determined directly. In specific embodiments, the primers are selected from primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 28 and 29 (LAMA1) and 34 and 35 (CDO1). In further embodiments, the probes are selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 30 (LAMA1) and 36 (CD01).

As for the methods, the kits are preferably employed in the context of faecal samples. Thus, the kit further comprises means for processing a faecal sample in certain embodiments. In view of the fact that the invention may be performed using only a small portion of the (total collected) faecal sample, as discussed herein (for example using as little as approximately 1g of stool to perform DNA extraction), the kits may incorporate a container suitably dimensioned to contain such a minimal/portioned sample. The container may be provided with a suitable collection device, adapted to retrieve and/or deposit an appropriate minimal/portioned sample (as described herein, of approximately 5, 4, 3, 2 or 1g or less). Suitable tubes and spoons for inclusion in such a kit are known in the art and commercially available. The spoons may be measuring spoons and may be integrated into the tubes in certain embodiments. The container may be dimensioned such that a suitable volume of homogenization buffer (as discussed herein, which discussion applies mutatis mutandis) may be added to the minimal/portioned sample added to the container.

The invention also provides a kit for any of:

(a) detecting a predisposition to, or the incidence of, colorectal cancer in a sample

(b) predicting the likelihood of successful treatment of colorectal cancer with a DNA demethylating agent and/or a DNA methyltransferase inhibitor and/or HDAC inhibitor

(c) predicting the likelihood of resistance to treatment of colorectal cancer with a DNA demethylating agent and/or DNA methyltransferase inhibitor and/or HDAC inhibitor; or (d) selecting a suitable treatment regimen for colorectal cancer comprising means for

detecting an epigenetic modification in at least one gene selected from GPNMB and MMP2 and means for processing a faecal sample.

As discussed herein, detection of the methylation status of these genes has been shown herein to provide a sensitive and specific means for detecting colorectal cancer in the context of faecal samples. In certain embodiments, the means for detecting an epigenetic modification in at least one gene selected from GPNMB and NMP1 comprise primers and/or probes which permit the methylation status of the at least one gene to be determined directly. In specific embodiments, the primers are selected from primers selected from primers comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 22 and 23 (GPNMB) and 31 and 32 (MMP2). In further embodiments, the probes are selected from probes comprising, consisting essentially of or consisting of the nucleotide sequences set forth as SEQ ID Nos: 24 (GPNMB) and 33 (MMP2).

In specific embodiments of these kits of the invention which involve means for processing a faecal sample, the means for processing a faecal sample comprises a sealable vessel for collection of a faecal sample. This vessel may be of a size and construction to permit a subject to directly defecate into the vessel. In view of the fact that the invention may be performed using only a small portion of the (total collected) faecal sample, as discussed herein (for example using as little as approximately 1 g of stool to perform DNA extraction), the kits may incorporate a container suitably dimensioned to contain such a minimal/portioned sample. The container may be provided with a suitable collection device, adapted to retrieve and/or deposit an appropriate minimal/portioned sample (as described herein, of approximately 5, 4, 3, 2 or 1g or less).

Suitable tubes and spoons for inclusion in such a kit are known in the art and commercially available. The spoons may be measuring spoons and may be integrated into the tubes in certain embodiments. The container may be dimensioned such that a suitable volume of homogenization buffer (as discussed herein, which discussion applies mutatis mutandis) may be added to the minimal/portioned sample added to the container.

In further embodiments, the means for processing a faecal sample comprises a homogenization buffer in certain embodiments. Suitable homogenization buffers are known in the art and commercially available. In related embodiments, the means for processing a faecal sample may (further) comprise reagents for extraction/isolation/concentration/purification of DNA from the faecal sample. As an example, the QIAamp DNA stool kit available from Qiagen includes suitable components for purification of total DNA from fresh or frozen faecal samples.

In order to permit determination of the methylation status of the panel of genes, the kits may further comprise a reagent which selectively modifies unmethylated cytosine residues in the DNA contained in the sample to produce detectable modified residues but which does not modify methylated cytosine residues. As discussed above, this permits sequence differences to be detected as an indication of the methylation status of the DNA. Any suitable reagent may be employed. Many examples are known in the art and commercially available. In certain embodiments, the reagent comprises a bisulphite reagent. More specifically, the bisulphite reagent may comprise, consist essentially of or consist of sodium bisulphite.

EXPERIMENTAL SECTION

Example 1

Sample collection and processing

A standardized multicenter screening trial (The Netherlands) was initiated in 2006. In this trial, non symptomatic subjects aged 50 or above are screened with colonoscopy, FOBT and real-time MSP using DNA from stool and blood. In addition, prospectively collected stool samples from multiple centers (Germany and The Netherlands) were used. In these trials, symptomatic patients, attending a Gastroenterology clinic and ultimately diagnosed with CRC, provided a stool sample for use in real-time MSP. From the ongoing trials 111 stool samples were available for the present study. 3 main categories of stool samples were used: 38 samples with no suspicious findings, 10 adenomas and 63 samples from patients covering all stages of CRC. After defecation in a special bucket, an iFOBT samples was

taken (Eiken device) and the patients subsequently added 250 ml of stool homogenization buffer (Amresco, Solon, Ohio, USA) to the sample. Samples were shipped to the laboratory and further processed within 72 hours after defecation. Stool homogenization buffer was added to a ratio 1 :7, and the samples were homogenized and aliquoted in portions of 32 ml.

iFOBT

The OC-Hemodia 'Eiken' was used to detect faecal occult blood when screening for early identification of colon cancer

DNA extraction from stool

Single aliquots (32 ml containing the equivalent of 4 g of stool) were centrifuged for 5 minutes at 2540 rcf at 20°C. The supernatant was retained and centrifuged a second time (10 minutes at 16500 rcf at 4°C). 22 ml of the supernatant obtained following the second centrifugation step was incubated with 5 µl Rnase A for 60 minutes at 37°C. Total DNA was then SodiumAcetate (pH 5.2) - isopropanol precipitated and washed with 70% ethanol. This "crude DNA" was resuspended in 4 ml 1x TE (pH 7.4). For set A (QIAamp DNA Stool Mini Kit): Add 1.6ml ASL Buffer to 300 µl "crude DNA" and vortex. The samples were centrifuged to pellet remaining stool particles. 1.4 ml of this supernatant was transferred into a new microcentrifuge tube. One InhibitEX Tablet was dissolved in each sample before a 1min incubation step to allow inhibitors to adsorb to the InhibitEX matrix. The samples were then centrifuged to pellet all remaining stool particles and inhibitors bound to InhibitEX matrix. The supernatant was transferred into a new microcentrifuge tube. 25 µl of proteinase K was added to 60OuI of sample supernatant before adding 600 µl of AL Buffer. The tubes are incubated at 70°C for 10 min. 600 µl of ethanol (96-100%) were added to the lysate, and mix by vortexing. Carefully apply 3x600 µl of lysate to QIAamp spin columns, centrifuge and discard the tube containing the filtrate. Carefully open the QIAamp spin column and add 500 µl of AW1 Buffer, centrifuge and discard the collection tube containing the filtrate. Carefully open the QIAamp spin column and add 500 µl of AW2 Buffer, centrifuge discard the collection tube containing the filtrate. Transfer the QIAamp spin column into a new microcentrifuge tube, open the QIAamp spin column, add 50 µl of AE Buffer onto the

membrane, incubate for 1 min at room temperature, and then centrifuge to elute DNA. For set B (QIAamp DNA Stool Midi Kit): Add 1.5ml ASL buffer to 2 ml of "Crude DNA". Add 1 inhibitEX Tablet to each sample and vortex IMMEDIATELY and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix. Centrifuge sample to pellet stool particles and inhibitors bound to InhibitEX matrix. Collect the supernatant and transfer it into a new 15 ml centrifuge tube, discard the pellet.

Add 150 µl proteinase K to 2 ml of the supernatant, mix well, add 2.4 ml of AL Buffer and vortex for 15 s. Incubate at 70°C for 10 min. Add 2 ml ethanol (96-100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking. In order to ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

Carefully transfer 3.3 ml of the sample onto the QIAamp Midi column placed in a 15 ml centrifuge tube and centrifuge at 1850 x g (3000rpm) for 3 min, and discard the filtrate. Place the QIAamp midi column back into the 15 ml centrifuge tube. Load the remainder of the sample onto the QIAamp Midi column, centrifuge again at 1850 x g (3000 rpm) for 3 minutes, and discard the filtrate. Place the QIAamp Midi column back into the 15 ml centrifuge tube, add 2 ml of AW1 Buffer, centrifuge at 4500 x g (5000 rpm) for 1 min, add 2ml of AW2 Buffer to the QIAamp Midi column, centrifuge at 4500 x g (5000 rpm) for 15 min, and discard the filtrate. Place the QIAamp Midi column in a clean 15 ml centrifuge tube, centrifuge for 1 min to dry the column, place the QIAamp Midi column in a clean 15 ml centrifuge tube, and discard the collection tube containing the filtrate.

Add 200 µl of AE Buffer directly onto the membrane of the QIAamp Midi column, incubate at room temperature for 5 min, and centrifuge at 4500 x g (5000 rpm) for 2 min. Reload the same 200µl onto the membrane, incubate at room temperature for 5 min, and centrifuge at 4500 x g (5000 rpm) for 2 min to elute the DNA.

DNA modification

1.5 µg of DNA were subjected to bisulfite modification in 96-wells format on a pipetting robot (Tecan), using the EZ-96DNA Methylation kit (Zymo Research), according to the manufacturer's protocol. Basically, aliquots of 45 µl were mixed with 5 µl of M-Dilution Buffer and incubated at 37°C for 15 minutes shaking at 1100 rpm. Then 100 µl of the diluted CT Conversion Reagent was added and samples were incubated at 70°C for 3 hours, shaking at 1100 rpm in the dark. After conversion, the samples were desalted by incubation on ice for 10 minutes and addition of 400 µl of M-Binding buffer. The samples were loaded on a Zymo-Spin I Column in a collection tube and after centrifugation washed with 200 µl of M-Wash Buffer. 200 µl of M-Desulphonation Buffer was put onto the column and incubated at room temperature for 15 minutes. After centrifugation of the columns, they were washed twice with 200 µl of M-Wash Buffer. Finally, the DNA was washed from the column in 50 µl Tris-HCI 1mM pH8.0 and stored at -80°C, until further processing.

DNA amplification

Real-time MSP was applied on a 7900HT fast real-time PCR system (Applied Biosystems). 5ul or 10ul of the modified DNA was added to a PCR mix (total volume 25 or 5OuI) containing buffer (16.6mM (NH4)2SO4, 67 mM Tris (pH 8.8), 6.7 mM MgCI2, 10 mM β-mercaptoethanol), dNTPs (5 mM), forward primer (6 ng), reverse primer (18 ng), molecular beacon (0.16 µM), BSA (0.1 µg), and Jumpstart DNA Taq polymerase (0.4 units; Sigma Aldrich). The primer sequences and molecular beacon sequences used for each of the genes are summarized in table 1. Cycle program used was as follows: 5 minutes 95°C, followed by 45 cycles of 30 seconds 95°C, 30 seconds 57°C (51 °C for APC), and 30 seconds 72°C, followed by 5 minutes 72°C. A standard curve (2x106 - 20 copies) was included to determine copy numbers of unknown samples by interpolation of their Ct values to the standard curve.

RESULTS

Table 1: Primers sequences and beacon sequences (for sample sets A and B)

Gene	Primer or probe	Nucleotide sequence (including labels on probe)	SEQ ID NO:
PHACTR	forward primer	5' - TTATTTTGCGAGCGGTTTC - 3	1
	reverse primer	5' - GAATACTCTAATTCCACGCGACT - 3'	2
	beacon	5'-FAM- CGACATGCGGGTTCGGTCGGCGCGGGGCATGTCG-3'-DABCYL	3
FOXE1	forward primer	5' - TTTGTTCGTTTTTCGATTGTTC - 3'	4
	reverse primer	5' - TAACGCTATAAAACTCCTACCGC - 3'	5
	beacon	5'-FAM- CGTCTCGTCGGGGTTCGGGCGTATTTTTTTAGGTAGGCGAGACG -3'-DABCYL	6
NDRG4	forward primer	5' - GTATTTTAGTCGCGTAGAAGGC - 3'	7
	reverse primer	5' - AATTTAACGAATATAAACGCTCGAC - 3'	8
	beacon	5'-FAM-CGACATGCCCGAACGAACCGCGATCCCTGCATGTCG-3'-DABCYL	9
GATA4	Forward primer	5'-AGGTTAGTTAGCGTTTTAGGGTC-3'	10
	Reverse primer	5'-ACGACGACGAAACCTCTCG-3'	11
	beacon	5'-FAM-CGACATGCCTCGCGACTCGAATCCCCGACCCAGCATGTCG-DABCYL-3'	12
OSMR	Forward primer	5' - TTTGGTCGGGGTAGGAGTAGC - 3'	13
	Reverse primer	5' - CGAACTTTACGAACGAACGAAC - 3'	14
	beacon	5'-FAM-CGACATGCCCGTACCCCGCGCGCAGCATGTCG-3'-DABCYL	15
SYNE1	Forward primer	5'-GTTGGGTTTTCGTAGTTTTGTAGATCGC-3'	16
	Reverse primer	5'-CTACGCCCAAACTCGACG-3'	17
	beacon	5'-FAM-CGACATGCCCCGCCCTATCGCCGAAATCGCATGTCG-DABCYL-3'	18
SFRP2	Forward primer	5' – GGGTCGGAGTTTTTCGGAGTTGCGC – 3'	19
	Reverse primer	5' – CCGCTCTCTTCGCTAAATACGACTCG – 3'	20
	beacon	5'-FAM- CGACATGCGGTGTTTCGTTTTTTCGCGTTTTAGTCGTCGGGCATGTCG - DABCYL-3'	21
GPNMB	Forward primer	5'-GGTCGTAGTCGTAGTCGGG-3'	22
	Reverse primer	5'-CCGCAAAAACCTAAAACGTAA-3'	23
	beacon	5'-FAM-CGACATGCGGTTTTTTGGGTCGGGGCGCGGCATGTCG-DABCYL-3'	24
SOX17	Forward primer	5'-GAGATGTTTCGAGGGTTGC-3'	25
	Reverse primer	5'-CCGCAATATCACTAAACCGA-3'	26
	beacon	5'-FAM-CGACATGCGTTCGTGTTTTGGTTTGTCGCGGTTTGGCATGTCG DABCYL-3'	27
LAMA1	Forward primer	5'-TTTTTAGATTTATCGAGTGGCG-3'	28
	Reverse primer	5'-CGAACTCACCTCTCTACCGAC-3'	29
	beacon	5'-CGACATGCCAAAAACACGCCCCCGCGCATGTCG-3'	30

MMP2	Forward primer	5'-TTCGGGTTATTAGCGTTTTTATC-3'	31
	Reverse primer	5'-ACTCCAACCAAACGACGAA-3'	32
	beacon	5'-FAM-CGACATCGTTGGTTCGGTGCGTGTGGTCGATGTCG-DABCYL-3'	33
CDO1	Forward primer	5'-AATTTGATTTGTGTGTGTATCGC-3'	34
	Reverse primer	5'-GAAACGTAAAAATATCGTCGCA-3'	35
	beacon	5'-FAM-CGACATGCGCGATTTCGGATTTATTGCGTTGTTAGGGCATGTCG-DABCYL-3'	36
TFPI2	Forward primer	5'-GTTCGTTGGGTAAGGCGTTC-3'	37
	Reverse primer	5'-CATAAAACGAACACCCGAACCG-3'	38
	Beacon	5'-FAM-CGACATGCACCGCGCACCTCCTCCCGCCAAGCATGTCG-DABCYL-3'	39

Performance of the individual methylation markers in fecal samples (set A)

Twelve methylation markers (GATA4, GPNMB, TFPI2, FoxE1 , SOX17, SYNE1, NDRG4, LAMA, MMP2, PHACTR ,OSMR and CDO1) were evaluated in fecal samples. Methylated copies of these genes were quantified in the available stool samples by real-time MSP on a 7900HT fast real-time PCR system (Applied Biosystems).

The individual performances of the twelve genes in fecal samples are shown in Table 2. The best performing gene in faecal samples from patients with CRC corresponded to NDRG4 with 61% sensitivity with a corresponding specificity of 100%.

Table 2: Performance of the individual methylation markers in faecal samples set A using a certain cutoff (gene copies).

Gene	cutoff	Spec (n=21)	Sens (n=23)
GATA4	10	90%	52%
GPNMB	100	100%	39%
TFPI2	100	100%	48%
FoxE1	5	100%	39%
SOX17	10	90%	35%
SYNE1	3	90%	43%
NDRG4	2	100%	61%
LAMA	40	100%	30%
MMP2	10	95%	39%
PHACTR	100	100%	43%
OSMR	1	95%	22%
CDO1	10	95%	13%

Performance of the 3 methylation marker combination in fecal samples set A

The performance of combinations of three methylation markers was also investigated. Methylated copies of the genes were quantified in the available stool samples by real-time MSP on a 7900HT fast real-time PCR system (Applied Biosystems). Table 3 shows the results and lists the cut-off (copies) applied. The best combinations had 100% specificity, 70% sensitivity for CRC.

Table 3: Performance of 3 methylation marker combinations in set A (top 3 rows give the cutoffs, empty cells denote that the gene was not used). A sample is scored as positive if any one marker is positive.

NDRG4 (copies)	2	2		2
FOXE1 (copies)	0	0	0	
PHACTR (copies)	100		100	100
Spec (n=21)	100%	100%	100%	100%
Sens (n=23)	70%	70%	52%	65%

Performance of FIT in fecal samples (set A)

FIT was evaluated in the same fecal samples. The performance in fecal samples is shown in Table 4. A specificity of 95% and sensitivity of 78% was obtained.

Table 4: Performance of FIT in fecal samples set A

	FIT
Cutoff (ng/ml)	100
Spec (n=21)	95%
Sens (n=23)	78%

Performance of the 3 methylation marker combination AND FIT in fecal samples set A

The performance of combination of the methylation markers with FIT was investigated. Methylated copies of the genes were quantified in the available stool samples by real-time MSP on a 7900HT fast real-time PCR system (Applied Biosystems). Table 5 shows the results and lists the cut-off (copies) applied.

Table 5: Performance of the methylation marker combinations in set A. A sample is scored as positive if any one marker is positive.

		Methylation marker only		Methylation markers plus FIT (100 ng/ml cutoff)	
Gene	cutoffs (copies)	Spec (n=21)	Sens (n=23)	Spec (n=21)	Sens (n=23)
GATA4	10	90%	52%	86%	87%
GPNMB	100	100%	39%	95%	87%
TFPI2	100	100%	48%	95%	91%
FoxE1	5	100%	39%	95%	87%
SOX17	10	90%	35%	86%	87%
SYNE1	3	90%	43%	86%	91%
NDRG4	2	100%	61%	95%	87%
LAMA	40	100%	30%	95%	83%
MMP2	10	95%	39%	90%	87%
PHACTR	100	100%	43%	95%	87%
OSMR	1	95%	22%	90%	83%
CDO1	10	95%	13%	90%	83%

Performance of the methylation marker combination AND FIT in fecal samples set A

The performance of a combination of the methylation markers with FIT was investigated, the results are shown in table 6.

Table 6: Performance of 3 methylation marker combinations in set A (top 4 rows give the cutoffs, empty cells denote that the gene was not used). A sample is scored as positive if any one marker is positive.

FIT (ng/ml)	100	100	100	100
NDRG4 (copies)	2	2		2
FOXE1 (copies)	0	0	0	
PHACTR (copies)	100		100	100
Spec (n=21)	95%	95%	95%	95%
Sens (n=23)	96%	96%	91%	91%

Performance of methylation markers in fecal samples (set B)

Seven methylation markers (NDRG4, FoxE1, PHACTR, GATA4, OSMR, SYNE1 , SFRP2) were evaluated in fecal samples. Methylated copies of these genes were quantified in the available stool samples by real-time MSP on a 7900HT fast real-time PCR system (Applied Biosystems). The individual performances of the 7 genes are shown in Table 7. The best performing gene in fecal samples from patients with CRC corresponded to PHACTR with 68% sensitivity with a corresponding specificity of 100%.

Table 7: Performance of the individual methylation markers in fecal samples set B

Gene	NDRG4	FoxE1	PHACTR	GATA4	OSMR	SYNE1	SFRP2
cutoff	2	0	100	10	1	3	2
Specificity	100%	94%	100%	100%	88%	100%	100%
Sensivity for advanced adenoma	0%	0%	10%	10%	20%	10%	10%
Sensivity for CRC	35%	55%	68%	43%	45%	65%	58%

Performance of FIT in fecal samples (set B)

FIT was evaluated in the same fecal samples. The performance in fecal samples is shown in Table 8.

Table 8: Performance of FIT in fecal samples set B

	FIT
Cutoff (ng/ml)	100
Specificity	100%
Sensivity for advanced adenoma	10%
Sensivity for CRC	78%

The FIT obtained a sensitivity of 78% and a corresponding specificity of 100%

Performance of the methylation markers and combinations thereof with or without FIT in fecal samples set B

The performance of combination of 3 methylation markers was investigated with or without FIT. Methylated copies of the genes were quantified in the available stool samples by real-time MSP on a 7900HT fast real-time PCR system (Applied Biosystems). Table 9 shows the results and lists the cut-off (copies) applied.

Table 9: Performance of the 3 methylation marker combinations in set B

FIT (ng/ml)	100	100	100	100				
NDRG4 (copies)	2	2		2	2	2		2
FOXE1 (copies)	0	0	0		0	0	0	
PHACTR (copies)	100		100	100	100		100	100
Spec (n=17)	94%	94%	94%	100%	94%	94%	94%	100%
Sens adv adenoma (n=10)	20%	20%	20%	20%	10%	0%	10%	10%
Sens CRC (n=40)	95%	88%	95%	95%	70%	58%	70%	68%

Use of FIT as first line screening and methylation as a second line confirmation assay

A simulation was made wherein the FIT test would be used a screening test and whereby a low cutoff of 10 ng/ml would be used to maximize the sensitivity. For samples with more than 10 ng/ml hemoglobin and less than 200 ng/ml the methylation test can be

used to increase the specificity for this subgroup. Samples are scored as positive if the FIT value is higher than 200 ng/ml or if one of the methylation markers is positive.

Table 10 shows the sensitivity and specificity of the FIT test as a first line test, as well as the overall sensitivity and specificity of the combination of FIT as first line and confirmation with "FIT > 200 OR a positive result on a methylation panel consisting of three genes".

Results for panel A when only applying the combination of FIT and methylation markers to samples having more than > 10 and less than 200 ng/ml hemoglobin.

Total number of tested with FIT = 44	Number of patients to be tested with the methylation panel = 5	FOXE1 (copies)	0
		NDRG4 (copies)	0
		PHACTR (copies)	50
		Spec (n=21)	95%
		Sens (n=23)	83%

Results for panel B when only applying the combination of FIT and methylation markers to samples having more than > 10 and less than 200 ng/ml hemoglobin.

Total number of tested with FIT = 67	Number of patients to be tested with the methylation panel = 12	FOXE1 (copies)	0
		NDRG4 (copies)	0
		PHACTR (copies)	50
		Spec (n=17)	94%
		Sens adv adenoma (n=10)	20%
		Sens CRC (n=40)	88%

Example 2

FIT and methylation testing on partial stool samples

In Example 1 homogenates of whole stool sample material were investigated. In this example, the inventor evaluated whether sufficient DNA material could be subtracted from partial stool samples allowing subsequent methylation analysis.

Sample collection and nucleic acid isolation

82 historical stool samples, collected between 2003 and 2008, were available for the present study. The samples were frozen down at the time of collection without addition of stabilizing buffer and stored at -20°C until further use.

In this study only a portion of the stool sample (≈ 1g) was investigated for further methylation testing. First, historical samples were thawed at the storage site and partial samples were taken (≈ 1g) using special designed stool tubes with integrated measuring spoons (Sarstedt). Each sample was then weighted and 2 volumes of stabilizing buffer (Diagn MoI Pathol; Volume 14, Number 3, September 2005) were added. Samples were stored at -80°C until the complete sample set was ready for transfer to the processing site.

DNA from these samples was prepared using the QiaAmp DNA stool minikit (Qiagen) following the same procedure as detailed in example 1 , sample set A. Adapted volumes were used for starting material and elution: 3 DNA isolations were done per stool sample, processing 250µl of homogenized stool material each, and elute in 75µl of buffer AE. At the end, eluates of the same sample were pooled and DNA was quantified using the Picogreen® dsDNA quantitation kit (Molecular Probes, #P7589) following the manufacturer's directions.

DNA modification and amplification

Samples were subjected to bisulfite modification using a commercially available kit (i.e. Zymo, #D5002) as discussed in example 1. Again, input material and elution volume were slightly adapted for this study: up to 2µg of input DNA was used and elution was done in 30µl Tris-HCI (1mM, pH 8.0); samples were stored at -80°C, until further processing.

Real-time MSP was performed as set out in example 1. DNA methylation patterns of the PHACTR3 gene were determined by chemical modification of unmethylated but not the methylated cytosines to uracil and subsequent PCR using primers specific for the methylated version, sequence details are provided in Table 1.

Results Methylation status for patients was studied in DNA extracted from partial stool samples derived from historical faecal collections. Average yield of DNA was 7µg (ranging from 0.3 to 27µg, SD 5µg) resulting in sufficient DNA material for subsequent bisulfite modification and real-time MSP procedure. Methylated copies of the PHACTR3 genes were quantified in the available stool portions. Performance of this methylation marker and combination with or without FIT were comparable with the above reported data from the prospectively collected whole stool sample set.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all embodiments described herein are considered to be broadly applicable and combinable with any and all other consistent embodiments, as appropriate.

CLAIMS

I claim:

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;

    b) in a sealable vessel, combining a first portion of the fecal sample with a stabilizing buffer, and sealing the sealable vessel; and

    c) in a sealable container, combining a second portion of the fecal sample with a solution that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container.

2. The method of claim 1, further comprising delivering the sealable vessel containing the first portion of the fecal sample and the stabilizing buffer and the sealable container containing the second portion of the fecal sample and the solution to a medical diagnostics laboratory.

3. The method of claim 1, wherein the fecal sample is collected at home by the human subject by defecation directly into the sealable vessel.

4. 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 vessel containing a first portion of a fecal sample and a stabilizing buffer; and

        ii) a sealed sealable container containing a second portion of a fecal sample and a solution that prevents denaturation or degradation of blood proteins found in a fecal sample,

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

b) extracting nucleic acid from the first portion of the fecal sample;

c) testing nucleic acid extracted from the first portion of the fecal sample for an amount of a human nucleic acid; and

d) testing the second portion of the fecal sample for an amount of a blood protein present in the second portion of the fecal sample.

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

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

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

8. The method of claim 6, wherein the epigenetic modification comprises aberrant methylation.

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

10. The method of claim 6, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.

11. The method of claim 10, wherein the gene is selected from the group consisting of *PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFPI2, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2,* and *CDO1*.

12. The method of claim 6, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfite ions under conditions wherein unmethylated cytosine is converted to uracil.

13. The method of claim 5, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the human gene.

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

15. The method of claim 4, wherein testing for an amount of a blood protein present in the second portion comprises testing for a concentration of hemoglobin in the second portion, wherein a concentration of hemoglobin is indicative of a presence of blood in the fecal sample.

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

17. The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 5 ng/ml.

18. The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 10 ng/ml.

19. The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 20 ng/ml.

20. The method of claim 15, wherein the second portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the second portion is at least 50 ng/ml.

ABSTRACT

A method of processing a fecal sample from a human subject comprising combining a first portion of a collected fecal sample with a stabilizing buffer, combining a second portion of the sample with a solution that prevents denaturation or degradation of blood proteins found in a fecal sample. Embodiments comprise testing nucleic acid extracted from the first portion of the fecal sample for an amount of a human nucleic acid, and testing the second portion of the fecal sample for the presence of human blood.

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