

De Luca also discloses testing a removed portion of the fecal sample for a blood protein, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Lapidus '650 in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* at paragraph 118.

'781 Patent, Claim 3, Third Limitation	Lapidus '650 in view of De Luca and Olson
c) extracting nucleic acid from the remaining portion of the fecal sample; and	Lapidus '650 discloses that “a whole stool may be used [for diagnostic assays]. The portion contains sufficient material to allow subsequent diagnostic assays to be performed.” Ex. 1003 at column 9, lines 21-24. Lapidus '650 discloses that the an illustrative “assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer. Known assay protocols, those disclosed herein or in copending application serial number Co-owned U.S. patent application, Ser. No. 08/700,583 or assays hereafter developed may be used in the practice of the invention. Non-limiting examples of useful known assay protocols include those disclosed in U.S. Pat. No. 5,137,806 (detection of sequences in selected DNA molecules), U.S. Pat. No. 5,348,855 (assay for nucleic acid sequences),

	U.S. Pat. No. 5,512,441 (detection of mutant alleles), U.S. Pat. No. 5,272,057 and U.S. Pat. No. 5,380,645 (RFLP analysis), U.S. Pat. No. 5,527,676 (detection of p53 gene sequences), U.S. Pat. No. 5,330,892 (detection of MCC gene sequences), U.S. Pat. No. 5,352,775 (detection of APC gene sequences), U.S. Pat. No. 5,532,108 (detection of DCC gene sequences), and in WO96/08514 (monoclonal antibodies against human colon carcinoma-associated antigens), the disclosures of each of which are incorporated by reference herein.” <i>Id.</i> at column 6, lines 6-28. Lapidus ‘650 discloses “[m]ethods of the invention are used to prepare a stool sample followed by detection of a deletion or other mutation in the p53 tumor suppressor gene.” ... DNA or RNA may optionally be isolated from the sample according to methods known in the art.” <i>Id.</i> at column 8, lines 39-50. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of
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	recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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Lapidus ‘650 discloses extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, it is logical that Lapidus ‘650 discloses a ‘remaining portion’ of the fecal sample that is left over after removal of a cross-sectional sample of the stool. Lapidus ‘650 discloses testing DNA and RNA from cellular components of the fecal sample. It is well known in the art that these tests include extracting nucleic acid. Ex. 1014 at paragraphs 124-125.

Even if Lapidus ‘650 is determined to not specifically teach removal to a separate sealable container, Olson removes this deficiency. As discussed *supra*, a person of ordinary skill in the art would have been motivated to add a preservative as a stabilizing buffer to the stool at the time of defecation as taught by Olson in order to prevent degradation of nucleic acids. *Id.* at paragraphs 127-128. To this end, Olson also discloses extracting nucleic acid from a fecal sample and indicates that addition of a stabilizing buffer to stool led to increased DNA assay

sensitivity. Therefore, one skilled in the art would recognize that Lapidus '650 in view of Olson disclose all of the requirements for the extracting step contained in the third limitation of claim 3 of the '781 Patent. *Id.* at paragraph 128.

'781 Patent, Claim 3, Fourth Limitation	Lapidus '650 in view of De Luca and Olson
d) testing the nucleic acid for an amount of a human nucleic acid.	Lapidus '650 discloses a protocol entitled "1. Method for Quantitation of Target and Reference Polynucleotides." Ex. 1003 at column 9, lines 63-65. Here, Lapidus '650 discloses "[e]nhanced quantification of binding events between hybridization probes and target or reference is accomplished by coupling hybridization probes to particles, such as beads (hybridization beads). In order to obtain a precise quantitative measure of the amount of a polynucleotide in a sample, hybridization beads are constructed such that each bead has attached thereto a single oligonucleotide probe." <i>Id.</i> at column 9, lines 66 – column 10, line 5. Olson discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of

	recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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Lapidus ‘650 discloses testing the nucleic acid for an amount of a human nucleic acid. Example 2 of Lapidus ‘650 discloses a procedure entitled “Method for Quantitation of Target and Reference Polynucleotides” and notes that a “precise quantitative measure of the amount of a polynucleotide in a sample” can be obtained. It is well known in the art that a ‘polynucleotide is a synonym for a ‘nucleic acid.’ Ex. 1014 at paragraph 132. Thus, Lapidus ‘650 indicates that nucleic acids can be quantitated according to its described assays. A person of ordinary skill in the art would understand that the described nucleic acids are human nucleic acid. *Id.*

Similarly, Olson also discloses testing the nucleic acid for an amount of a human nucleic acid. For instance, Table 1 of Olson lists “quantification of recoverable human DNA” in samples incubated with stabilization buffer. Therefore, a person of ordinary skill in the art would recognize that Lapidus ‘650 in view of Olson discloses all of the requirements for the

testing step contained in the fourth limitation of claim 3 of the '781 Patent. *Id.* at paragraphs 128-129.

In summary, Lapidus '650 in view of De Luca and Olson therefore disclose the additional limitations of claim 3 of the '781 Patent. Because the other limitations of claim 3 are obvious for the reasons explained above for claim 1, claim 3 is also obvious over Lapidus '650 in view of De Luca and Olson.

7. Ahlquist in view of De Luca and Olson Renders Claim 3 Obvious

'781 Patent, Claim 3, Preamble	Ahlquist in view of De Luca and Olson
3. A method of processing a fecal sample, the method comprising:	Ahlquist discloses “[p]atients collected 3 stools by using plastic buckets mounted to the toilet seat. Promptly after each individual collection, patients smeared stool onto both windows of their Hemocult and HemocultSensa cards and then express-shipped smeared cards and the whole stool (sealed in a bucket in an insulated container cooled with ice packs) to the Mayo Clinic in Rochester, Minnesota. Ex. 1004 at page 4, at ‘Stool Collection and Processing’ heading.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Ahlquist discloses this limitation because Ahlquist discloses a stool (a fecal sample) collected by a patient into a plastic bucket that is transported to the Mayo Clinic for analysis. Furthermore, as discussed *supra*, Ahlquist discloses the “method of processing” of independent claim 1 from which claim 3 depends, should the preamble of claim 1 be determined to be a limitation.

'781 Patent, Claim 3, First Limitation	Ahlquist in view of De Luca and Olson
a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising: i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer, the pair of portions obtained by the method of claim 1;	Ahlquist discloses “[p]atients collected 3 stools by using plastic buckets mounted to the toilet seat. Promptly after each individual collection, patients smeared stool onto both windows of their Hemoccult and HemoccultSensa cards and then express-shipped smeared cards and the whole stool (sealed in a bucket in an insulated container cooled with ice packs) to the Mayo Clinic in Rochester, Minnesota. Ex. 1004 at page 4, at ‘Stool Collection and Processing’ heading. Ahlquist discloses that “[w]e did not evaluate fecal immunochemical blood tests, and such tests may detect more adenomas than do guaiac tests, because of differences in relative detection rates. <i>Id.</i> at page 9, second full paragraph. (citations omitted). De Luca discloses “an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, <i>Helicobacter pylori</i> and the like. Ex. 1006 at [0001]. De Luca discloses “an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician.” <i>Id.</i> at [0015].

	Further, De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. De Luca discloses that on the extraction tube, “[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap.” <i>Id.</i> at [0032]. Ahlquist states that “[t]echnical advances could improve DNA assay sensitivity” on the stools and that “[m]ethods to increase stool DNA capture are needed, and some have been reported.” <i>Id.</i> at page 9, third full paragraph (citations omitted). Ahlquist states that its authors and other researchers “have shown that adding a preservative to stool at the time of defecation can prevent degradation of long DNA.” <i>Id.</i> Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments
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	associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose obtaining a pair of portions of a fecal sample collected from a human subject. As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the pair of portions are obtained by the method of claim 1. Accordingly, a person of ordinary skill in the art would recognize that Ahlquist in view of De

Luca and Olson discloses all of the requirements for the obtaining step contained in the first limitation of claim 3 of the '781 Patent. Ex. 1014 at paragraphs 112-113.

'781 Patent, Claim 3, Second Limitation	Ahlquist in view of De Luca and Olson
b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;	Ahlquist discloses for occult blood testing that “[t]he manufacturer that developed the Hemoccult and HemoccultSensa cards, without rehydration, trained technicians on-site at the Mayo Clinic. As recommended by the manufacturer, the technicians added the catalyst solution to cards stored at ambient temperature within 48 to 72 hours of collection. We defined a spreading (enlarging) blue color in 60 seconds in any window of the cards as a positive result and any other result as negative.” Ex. 1004 at page 5, at heading ‘Occult Blood Testing.’ Ahlquist discloses that it “We compared fecal blood results from 3 stools per patient with stool DNA on 1 stool. Experienced technicians performed stool DNA and occult blood testing in separate central laboratories without knowledge of clinical findings or the results of other tests.” <i>Id.</i> at page 3, at heading ‘Design.’ Further, Ahlquist discloses that “[d]etection sensitivities for the 290 screen-relevant neoplasms found among all 3764 evaluable participants were 10% (95% CI, 7% to 13%) with Hemoccult and 18% (CI, 13% to 22%) with HemoccultSensa (P <

	0.001). Based on all 3474 participants without screenrelevant neoplasia, the Hemoccult specificity of 98% (CI, 98% to 99%) was slightly higher than that of HemoccultSensa (97% [CI, 96% to 97%]) ($P < 0.001$). Hemoccult and HemoccultSensa positivity rates for the 39 patients with colorectal cancer or high-grade dysplasia were 33% (CI, 19% to 48%) and 44% (CI, 28% to 59%), respectively ($P = 0.100$). For the 251 patients with adenomas 1 cm or larger, the positivity rates were 6% (CI, 3% to 9%) versus 14% (CI, 9% to 18%) ($P = 0.001$).” <i>Id.</i> at pages 6-y, under heading ‘Occult Blood Testing: Hemoccult versus HemoccultSensa.’ De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i>
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	at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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Ahlquist discloses testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion. As discussed *supra*, Ahlquist discloses that the patients collected stool and then “smear[ed] stool” onto Hemocult and HemocultSensa cards. This smeared stool sample corresponds to the ‘removed portion’ of the fecal sample. Further, Ahlquist discloses that occult blood testing was performed on the Hemocult and HemocultSensa cards. It is well known in the art that occult blood testing refers to analyzing for the presence of blood proteins such as hemoglobin. Ex. 1014 at paragraphs 119-120.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for a blood protein by utilizing the fecal immunochemical blood tests taught by De Luca and as suggested by Ahlquist instead of the guaiac-based Hemocult and HemocultSensa tests of Ahlquist. *Id.* To this end, De Luca also discloses testing a removed portion of the fecal sample for a blood protein, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Ahlquist in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the ‘781 Patent. *Id.*

‘781 Patent, Claim 3, Third Limitation	Ahlquist in view of De Luca and Olson
c) extracting nucleic acid from the remaining portion of the fecal sample; and	Ahlquist discloses that “[a]ll assays were polymerase chain reaction–based and were run at EXACT Sciences. Stool DNA test 1 was performed as described in Imperiale and colleagues’ study (21). The marker panel for

	SDT-1 included 21 tumor-specific point mutations (3 on the Kras gene, 10 on the APC gene, and 8 on the p53 gene); the microsatellite-instability marker BAT-26; and long DNA, a marker for delayed apoptosis, which is characteristic of exfoliated neoplastic colonocytes (12). For SDT-2, sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays. We defined any positive component marker result according to the manufacturer's preestablished criteria as a positive test result. Ex. 1004, pages 4-5, at 'DNA testing' heading. Ahlquist states that "[t]echnical advances could improve DNA assay sensitivity" on the stools and that "[m]ethods to increase stool DNA capture are needed, and some have been reported." <i>Id.</i> at page 9, third full paragraph (citations omitted). Ahlquist states that its authors and other researchers "have shown that adding a preservative to stool at the time
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	of defecation can prevent degradation of long DNA.” <i>Id.</i> Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer
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	to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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Ahlquist discloses extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, Ahlquist discloses that the patients collected stool and that rest of the stool in the plastic bucket (e.g., not including the smeared stool) corresponds to the ‘remaining portion’ of the fecal sample in independent claim 1.

Further, Ahlquist discloses that one or two “Stool DNA tests” were performed on stool via polymerase chain reaction and that sequence-specific DNA markers were detected. It is well known in the art that these tests include extracting nucleic acid. Ex. 1014 at paragraph 129.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to add a preservative as a stabilizing buffer to the stool at the time of defecation as taught by Olson and as suggested by Ahlquist in order to prevent degradation of nucleic acids. *Id.* To this end, Olson also discloses extracting nucleic acid from a fecal sample and indicates that addition of a stabilizing buffer to stool led to increased DNA assay sensitivity. Therefore, one skilled in the art would recognize that Ahlquist in view of Olson disclose all of the requirements for the extracting step contained in the third limitation of claim 3 of the ‘781 Patent. *Id.*

‘781 Patent, Claim 3, Fourth Limitation	Ahlquist in view of De Luca and Olson
d) testing the nucleic acid for an amount of a human nucleic acid.	Ahlquist discloses that “[a]ll assays were polymerase chain reaction–based and were run at EXACT Sciences. Stool DNA test 1 was performed as described in Imperiale and colleagues’ study (21). The marker panel for SDT-1 included 21 tumor-specific point mutations (3 on the Kras gene, 10 on the APC gene, and 8 on the p53 gene); the microsatellite-instability marker BAT-26; and long DNA, a marker for delayed apoptosis, which is characteristic of exfoliated neoplastic

	colonocytes (12). For SDT-2, sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays. We defined any positive component marker result according to the manufacturer's preestablished criteria as a positive test result. Ex. 1004, pages 4-5, at 'DNA testing' heading. Ahlquist states that "[t]echnical advances could improve DNA assay sensitivity" on the stools and that "[m]ethods to increase stool DNA capture are needed, and some have been reported." <i>Id.</i> at page 9, third full paragraph (citations omitted). Ahlquist states that its authors and other researchers "have shown that adding a preservative to stool at the time of defecation can prevent degradation of long DNA." <i>Id.</i> Olson discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific
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	mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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Ahlquist discloses testing the nucleic acid for an amount of a human nucleic acid. In particular, Ahlquist discloses testing of nucleic acid via two stool DNA tests (i.e., SDT-1 and SDT-2) and corresponds the amount of identified nucleic acid with detection of neoplasm in the

human patients. Amongst others, Ahlquist discloses testing for human nucleic acids such as *K-ras*, *APC*, and *p53*.

Similarly, Olson also discloses testing the nucleic acid for an amount of a human nucleic acid. For instance, Table 1 of Olson lists “quantification of recoverable human DNA” in samples incubated with stabilization buffer. Therefore, a person of ordinary skill in the art would recognize that Ahlquist in view of Olson discloses all of the requirements for the testing step contained in the fourth limitation of claim 3 of the ‘781 Patent. Ex. 1014 at paragraph 136.

In summary, Ahlquist in view of De Luca and Olson therefore disclose the additional limitations of claim 3 of the ‘781 Patent. Because the other limitations of claim 3 are obvious for the reasons explained above for claim 1, claim 3 is also obvious over Ahlquist in view of De Luca and Olson.

8. O’Neill in view of De Luca and Olson Renders Claim 3 Obvious

‘781 Patent, Claim 3, Preamble	O’Neill in view of De Luca and Olson
3. A method of processing a fecal sample, the method comprising:	O’Neill discloses that “[i]f 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.” <i>Id.</i> at page 8, lines 7-11.

Assuming, without conceding, that the preamble of claim 3 is a limitation, O’Neill discloses this limitation because O’Neill discloses a stool (a fecal sample) collected by a patient into a container that is transported to a clinic for analysis. Furthermore, as discussed *supra*, O’Neill discloses the “method of processing” of independent claim 1 from which claim 3 depends, should the preamble of claim 1 be determined to be a limitation.

'781 Patent, Claim 3, First Limitation	O'Neill in view of De Luca and Olson
a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising: i) a sealed sealable container containing a removed portion of a fecal sample and a buffer; and ii) a sealed sealable collection vessel containing a remaining portion of a fecal sample and a stabilizing buffer, the pair of portions obtained by the method of claim 1;	O'Neill discloses "a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools." Ex. 1005 at page 1, lines 5-7. O'Neill discloses "a method of removing cellular material from the surface of a faecal stool" in a container that comprising the steps of "introducing wash liquid into the container whilst maintaining the stool at a temperature below its gel freezing point" and then "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." <i>Id.</i> at page 3, lines 16-27. Thereafter, O'Neill further discloses "[t]he 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." <i>Id.</i> at page 6, line 31 – page 7, line 4. Finally, O'Neill discloses that the wash liquid can "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.” <i>Id.</i> at page 14, lines 27-33. De Luca discloses “an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, <i>Helicobacter pylori</i> and the like. Ex. 1006 at [0001]. De Luca discloses “an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician.” <i>Id.</i> at [0015]. Further, De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. De Luca discloses that on the extraction tube, “[a]n annular seal 55 is
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	provided between the knurled part 44 and the threaded part 45 of the top cap.” <i>Id.</i> at [0032]. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer
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	to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, O'Neill in view of De Luca and Olson disclose obtaining a pair of portions of a fecal sample collected from a human subject. As discussed *supra*, O'Neill in view of De Luca and Olson disclose the pair of portions are obtained by the method of claim 1. Accordingly, a person of ordinary skill in the art would recognize that O'Neill in view of De Luca and Olson discloses all of the requirements for the obtaining step contained in the first limitation of claim 3 of the '781 Patent. Ex. 1014 at paragraph 113.

'781 Patent, Claim 3, Second Limitation	O'Neill in view of De Luca and Olson
b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;	O'Neill discloses "a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools." Ex. 1005 at page 1, lines 5-7. O'Neill discloses that "[e]xfoliated 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." <i>Id.</i> at page 1, lines 15-24.

	De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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O'Neill discloses testing the removed portion of cellular material from the fecal sample for various components, such as DNA, RNA, and proteins. It is well known in the art that testing such components from fecal samples could include occult blood testing, fecal immunochemical blood tests, and the like for evaluating colorectal cancer in patients. Ex. 1014 at paragraphs 121-122.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for a blood protein by utilizing the

fecal immunochemical blood tests taught by De Luca. Ex. 1014 at paragraphs 117. To this end, De Luca also discloses testing a removed portion of the fecal sample for a blood protein, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that O'Neill in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* at paragraphs 121-122.

'781 Patent, Claim 3, Third Limitation	O'Neill in view of De Luca and Olson
c) extracting nucleic acid from the remaining portion of the fecal sample; and	O'Neill discloses “a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools.” Ex. 1005 at page 1, lines 5-7. O'Neill discloses that “[e]xfoliated 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.” <i>Id.</i> at page 1, lines 15-24. Further, O'Neill discloses that “[t]he 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.” <i>Id.</i> at page 16, lines 7-19. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room
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	temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, O’Neill discloses that the patients collected stool in a container and that after removal of some cellular material, a large mass of stool remains in the container thus corresponding to the ‘remaining portion’ of the fecal sample in independent claim 1. O’Neill discloses testing DNA and RNA from cellular components of the fecal sample and discloses detailed examples of DNA analyses, such as fluorimetric DNA assay was performed with a TECAN™ Spectrafluor Plus using PicoGreen (Molecular Probes™) to yield the stool DNA index (SDNAI). It is well known in the art that these tests include extracting nucleic acid. Ex. 1014 at paragraph 130.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to add a preservative as a stabilizing buffer to the stool at the time of defecation as taught by Olson in order to prevent degradation of nucleic acids. *Id.* at paragraphs 127-128. To this end, Olson also discloses extracting nucleic acid from a fecal sample and indicates that addition of a stabilizing buffer to stool led to increased DNA assay sensitivity. Therefore, one skilled in the art would recognize that O’Neill in view of Olson disclose all of the requirements for the extracting step contained in the third limitation of claim 3 of the ‘781 Patent. *Id.* at paragraph 130.

'781 Patent, Claim 3, Fourth Limitation	O'Neill in view of De Luca and Olson
d) testing the nucleic acid for an amount of a human nucleic acid.	O'Neill discloses "a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools." Ex. 1005 at page 1, lines 5-7. O'Neill discloses that "[e]xfoliated 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." <i>Id.</i> at page 1, lines 15-24. Further, O'Neill discloses that "[t]he 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.” <i>Id.</i> at page 16, lines 7-19. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).”
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	<i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, O’Neill discloses testing DNA and RNA from cellular components of the fecal sample. It is well known in the art that these tests include testing the nucleic acid for an amount of a human nucleic acid. Ex. 1014 at paragraphs 137-138.

Similarly, Olson also discloses testing the nucleic acid for an amount of a human nucleic acid. For instance, Table 1 of Olson lists “quantification of recoverable human DNA” in samples incubated with stabilization buffer. Therefore, a person of ordinary skill in the art would recognize that O’Neill in view of Olson discloses all of the requirements for the testing step contained in the fourth limitation of claim 3 of the ‘781 Patent. *Id.*

In summary, O’Neill in view of De Luca and Olson therefore disclose the additional limitations of claim 3 of the ‘781 Patent. Because the other limitations of claim 3 are obvious for the reasons explained above for claim 1, claim 3 is also obvious over O’Neill in view of De Luca and Olson.

D. Dependent Claim 4

1. Lapidus ‘178 Anticipates Claim 4

‘781 Patent, Claim 4	Lapidus ‘178
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus ‘178 describes “methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood.” Ex. 1002 at column 2, lines 59-63. Further, Lapidus ‘178 describes “DNA or RNA may be isolated from the homogenate using methods known

	in the art. Subsequent tests may be performed on the isolated DNA and RNA.” <i>Id.</i> at column 9, lines 47-49.
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Lapidus ‘178 describes testing the nucleic acid for determining expression from a human gene. Again, Lapidus ‘178 describes isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of measuring expression from a human gene. Ex. 1014 at paragraph 148. Thus, Lapidus ‘178 describes determination of expression of a human gene via testing of a nucleic acid.

Lapidus ‘178 therefore describes the additional limitations of claim 4 of the ‘781 Patent. Because the other limitations of claim 4 are anticipated for the reasons explained above for claims 1 and 3, claim 4 is also anticipated by Lapidus ‘178.

2. Lapidus ‘650 Anticipates Claim 4

‘781 Patent, Claim 4	Lapidus ‘650
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus ‘650 describes “methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood.” Ex. 1003 at column 2, lines 50-54. Further, Lapidus ‘650 describes “DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA.” <i>Id.</i> at column 8, lines 22-25.

Lapidus ‘650 describes testing the nucleic acid for determining expression from a human gene. Again, Lapidus ‘650 describes isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of

measuring expression from a human gene. Ex. 1014 at paragraph 148. Thus, Lapidus '650 describes determination of expression of a human gene via testing of a nucleic acid.

Lapidus '650 therefore describes the additional limitations of claim 4 of the '781 Patent. Because the other limitations of claim 4 are anticipated for the reasons explained above for claims 1 and 3, claim 4 is also anticipated by Lapidus '650.

3. Lapidus '178 Renders Claim 4 Obvious

'781 Patent, Claim 4	Lapidus '178
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus '178 discloses "methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood." Ex. 1002 at column 2, lines 59-63. Further, Lapidus '178 discloses "DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA." <i>Id.</i> at column 9, lines 47-49.

In the alternative to the assertion that Lapidus '178 anticipates claim 4, Lapidus '178 further renders claim 4 obvious.

Lapidus '178 discloses testing the nucleic acid for determining expression from a human gene. Again, Lapidus '178 discloses isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of measuring expression from a human gene. Ex. 1014 at paragraph 148. Thus, Lapidus '178 discloses determination of expression of a human gene via testing of a nucleic acid.

Lapidus '178 therefore discloses the additional limitations of claim 4 of the '781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claims 1 and 3, claim 4 is also obvious over Lapidus '178.

4. Lapidus '650 Renders Claim 4 Obvious

'781 Patent, Claim 4	Lapidus '650
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus '650 discloses “methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood.” Ex. 1003 at column 2, lines 50-54. Further, Lapidus '650 describes “DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA.” <i>Id.</i> at column 8, lines 22-25.

In the alternative to the assertion that Lapidus '650 anticipates claim 4, Lapidus '650 further renders claim 4 obvious.

Lapidus '650 discloses testing the nucleic acid for determining expression from a human gene. Again, Lapidus '650 discloses isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of measuring expression from a human gene. Ex. 1014 at paragraph 148. Thus, Lapidus '650 discloses determination of expression of a human gene via testing of a nucleic acid.

Lapidus '650 therefore discloses the additional limitations of claim 4 of the '781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claims 1 and 3, claim 4 is also obvious over Lapidus '650.

5. Lapidus '178 in view of De Luca and Olson Renders Claim 4 Obvious

'781 Patent, Claim 4	Lapidus '178 in view of De Luca and Olson
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus '178 discloses “methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood.” Ex. 1002 at column 2, lines 59-63. Further, Lapidus '178 discloses “DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA.” <i>Id.</i> at column 9, lines 47-49. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the

	samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 3. As discussed *supra*, Lapidus ‘178 discloses isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of measuring expression from a human gene. Ex. 1014 at paragraph 148. Similarly, Olson also discloses testing the nucleic acid for human DNA. It is well known in the art that these tests include testing the nucleic acid comprises determining expression from a human gene. *Id.*

Lapidus ‘178 in view of De Luca and Olson therefore discloses the additional limitations of claim 4 of the ‘781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claim 3, claim 4 is also obvious over Lapidus ‘178 in view of De Luca and Olson.

6. Lapidus '650 in view of De Luca and Olson Renders Claim 4 Obvious

'781 Patent, Claim 4	Lapidus '650 in view of De Luca and Olson
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Lapidus '650 discloses “methods of the invention comprise detection of characteristics of such transformed cells, including, for example, mutations, proteins expressed uniquely or in altered amounts in transformed cells, and blood.” Ex. 1003 at column 2, lines 50-54. Further, Lapidus '650 describes “DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA.” <i>Id.</i> at column 8, lines 22-25. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the

	samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 3. As discussed *supra*, Lapidus ‘650 discloses isolation of nucleic acids (e.g., DNA or RNA) and subsequent evaluation. It is well known in the art that testing of RNA is a definition of measuring expression from a human gene. Ex. 1014 at paragraph 148. Similarly, Olson also discloses testing the nucleic acid for human DNA. It is well known in the art that these tests include testing the nucleic acid comprises determining expression from a human gene. *Id.*

Lapidus ‘650 in view of De Luca and Olson therefore discloses the additional limitations of claim 4 of the ‘781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claim 3, claim 4 is also obvious over Lapidus ‘650 in view of De Luca and Olson.

7. Ahlquist in view of De Luca and Olson Renders Claim 4 Obvious

'781 Patent, Claim 4	Ahlquist in view of De Luca and Olson
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	Ahlquist discloses that “[a]ll assays were polymerase chain reaction–based and were run at EXACT Sciences. Stool DNA test 1 was performed as described in Imperiale and colleagues’ study (21). The marker panel for SDT-1 included 21 tumor-specific point mutations (3 on the Kras gene, 10 on the APC gene, and 8 on the p53 gene); the microsatellite-instability marker BAT-26; and long DNA, a marker for delayed apoptosis, which is characteristic of exfoliated neoplastic colonocytes (12). For SDT-2, sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays. We defined any positive component marker result according to the manufacturer’s preestablished criteria as a positive test result. Ex. 1004, pages 4-5, at ‘DNA testing’ heading. Further, Ahlquist discloses “we examined SDT-2 (which targets

	a potentially more informative marker panel) in a more limited manner. Stool DNA test 2 had significantly better neoplasm detection rates than did the fecal blood tests or SDT-1. We accounted for the higher detection rates of SDT-2 by better detection of advanced adenomas, a finding of particular importance for cancer prevention.” <i>Id.</i> at page 9, first full paragraph.
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 3. Further, Ahlquist discloses testing the nucleic acid comprises determining expression from a human gene. In particular, Ahlquist discloses testing of nucleic acid via two stool DNA tests (i.e., SDT-1 and SDT-2) that identify human genes such as the Kras gene, the APC gene, and the p53 gene. Determining expression of these cancer-causing genes is evident given the identification of neoplasms in the patients and correlation to the human genes. Ex. 1014 at paragraphs 151 and 153.

Ahlquist in view of De Luca and Olson therefore discloses the additional limitations of claim 4 of the ‘781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claim 3, claim 4 is also obvious over Ahlquist in view of De Luca and Olson.

8. O’Neill in view of De Luca and Olson Renders Claim 4 Obvious

‘781 Patent, Claim 4	O’Neill in view of De Luca and Olson
4. The method of claim 3, wherein testing the nucleic acid comprises determining expression from a human gene.	O’Neill discloses “a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools.” Ex. 1005 at page 1, lines 5-7. O’Neill discloses that “[e]xfoliated 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.” <i>Id.</i> at page 1, lines 15-24. Olson discloses development of “a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC).” Ex. 1007 at Abstract. Olson discloses that “[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one
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	exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3).” <i>Id.</i> at page 186, first column, second full paragraph. In conclusion, Olson states that “DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. <i>Id.</i> at Abstract.
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As discussed *supra*, O’Neill in view of De Luca and Olson disclose the limitations of claim 3. As discussed *supra*, O’Neill discloses testing DNA and RNA from cellular components of the fecal sample including “procedures for qualitative and/or quantitative analysis of, for example, DNA alterations, gene expression and enzyme activity.” Similarly, Olson also discloses testing the nucleic acid for human DNA. It is well known in the art that these tests include testing the nucleic acid comprises determining expression from a human gene. Ex. 1014 at paragraphs 152-153.

O’Neill in view of De Luca and Olson therefore discloses the additional limitations of claim 4 of the ‘781 Patent. Because the other limitations of claim 4 are obvious for the reasons explained above for claim 3, claim 4 is also obvious over O’Neill in view of De Luca and Olson.

E. Dependent Claim 5

1. Lapidus ‘178 in view of De Luca, Olson, and Leung Renders Claim 5 Obvious

‘781 Patent, Claim 5	Lapidus ‘178 in view of De Luca, Olson, and Leung
5. The method of claim 4, wherein determining expression from the human gene comprises testing the nucleic acid for the	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with

presence of human DNA having an epigenetic modification.	colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 4.

The ‘781 patent defines “epigenetic modification” such that it includes “any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene.” ‘781 patent at column 12, lines 35-39. Furthermore, the ‘781 patent states that “[i]n specific embodiments, the epigenetic modification is methylation.” *Id.* at column 12, line 46-47.

As discussed *supra*, Lapidus ‘178 and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, Lapidus ‘178/De Luca/Olson does not specifically disclose testing the nucleic acid for the presence of human DNA having an epigenetic modification.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect an epigenetic modification (methylation) of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect an epigenetic modification of DNA in feces as disclosed in claim 5. Ex. 1014 at paragraphs 164-165. A skilled person would have a reasonable expectation of success to detect an epigenetic modification of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 5. Because the other limitations of claim 5 are obvious for the reasons explained above for claim 4, claim 5 is also obvious over Lapidus ‘178 in view of De Luca, Olson, and Leung.

2. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 5 Obvious

‘781 Patent, Claim 5	Lapidus ‘650 in view of De Luca, Olson, and Leung
5. The method of claim 4, wherein determining expression from the human gene comprises testing the nucleic acid for the presence of human DNA having an epigenetic modification.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 4.

The ‘781 patent defines “epigenetic modification” such that it includes “any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene.” ‘781 patent at column 12, lines 35-39. Furthermore, the ‘781 patent states that “[i]n specific embodiments, the epigenetic modification is methylation.” *Id.* at column 12, line 46-47.

As discussed *supra*, Lapidus ‘650 and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, Lapidus ‘650/De Luca/Olson does not specifically disclose testing the nucleic acid for the presence of human DNA having an epigenetic modification.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect an epigenetic modification (methylation) of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect an epigenetic modification of DNA in feces as disclosed in claim 5. Ex. 1014 at paragraphs 164-165. A skilled person would have a reasonable expectation of success to detect an epigenetic modification of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 5. Because the other limitations of claim 5 are obvious for the reasons explained above for claim 4, claim 5 is also obvious over Lapidus ‘650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca and Olson Renders Claim 5 Obvious

'781 Patent, Claim 5	Ahlquist in view of De Luca and Olson
5. The method of claim 4, wherein determining expression from the human gene comprises testing the nucleic acid for the presence of human DNA having an epigenetic modification.	Ahlquist discloses for evaluation of the second stool DNA test (SDT-1), “sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays.” Ex. 1004, page 4 at ‘DNA Testing’ heading.

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 4.

The ‘781 patent defines “epigenetic modification” such that it includes “any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene.” ‘781 patent at column 12, lines 35-39. Furthermore, the ‘781 patent states that “[i]n specific embodiments, the epigenetic modification is methylation.” *Id.* at column 12, line 46-47.

Ahlquist teaches determining expression from a human gene, wherein the nucleic acid is tested for the presence of human DNA having an epigenetic modification. In particular, Ahlquist discloses that assays for “methylation of the vimentin gene” were undertaken. A person of ordinary skill in the art would recognize that the vimentin gene is a human gene and that

evaluation of its methylation would meet the definition of testing for an epigenetic modification as defined by the '781 patent. Ex. 1014 at paragraphs 163 and 165.

Ahlquist in view of De Luca and Olson therefore discloses the additional limitations of claim 5 of the '781 Patent. Because the other limitations of claim 5 are obvious for the reasons explained above for claim 4, claim 5 is also obvious over Ahlquist in view of De Luca and Olson.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 5 Obvious

'781 Patent, Claim 5	O'Neill in view of De Luca, Olson, and Leung
5. The method of claim 4, wherein determining expression from the human gene comprises testing the nucleic acid for the presence of human DNA having an epigenetic modification.	Leung discloses "detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps." Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 4.

The '781 patent defines "epigenetic modification" such that it includes "any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene." '781 patent at column 12, lines 35-39. Furthermore, the '781 patent states that "[i]n specific embodiments, the epigenetic modification is methylation." *Id.* at column 12, line 46-47.

As discussed *supra*, O'Neill and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, O'Neill/De Luca/Olson does not specifically disclose testing the nucleic acid for the presence of human DNA having an epigenetic modification.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect an epigenetic modification (methylation) of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect an epigenetic modification of DNA in feces as

disclosed in claim 5. Ex. 1014 at paragraphs 164-165. A skilled person would have a reasonable expectation of success to detect an epigenetic modification of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 5. Because the other limitations of claim 5 are obvious for the reasons explained above for claim 4, claim 5 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

F. Dependent Claim 6

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 6 Obvious

'781 Patent, Claim 6	Lapidus '178 in view of De Luca, Olson, and Leung
6. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises measuring an amount of a methylated human DNA.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Lapidus '178 in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of a methylated human DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of a methylated human DNA in feces as disclosed in claim 6. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to measure an amount of a methylated human DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 6. Because the other limitations of claim 6 are obvious for the reasons explained above for claim 5, claim 6 is also obvious over Lapidus '178 in view of De Luca, Olson, and Leung.

2. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 6 Obvious

‘781 Patent, Claim 6	Lapidus ‘650 in view of De Luca, Olson, and Leung
6. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises measuring an amount of a methylated human DNA.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Lapidus ‘650 in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of a methylated human DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of a methylated human DNA in feces as disclosed in claim 6. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to measure an amount of a methylated human DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 6. Because the other limitations of claim 6 are obvious for the reasons explained above for claim 5, claim 6 is also obvious over Lapidus ‘650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca and Olson Renders Claim 6 Obvious

‘781 Patent, Claim 6	Ahlquist in view of De Luca and Olson
6. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises	Ahlquist discloses for evaluation of the second stool DNA test (SDT-1), “sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described

measuring an amount of a methylated human DNA.	by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays.” Ex. 1004, page 4 at ‘DNA Testing’ heading.
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 5. Further, Ahlquist teaches determining expression from a human gene, wherein the nucleic acid is tested for the presence of human DNA having an epigenetic modification by measuring an amount of a methylated human DNA. In particular, Ahlquist discloses that assays for “methylation of the vimentin gene” were undertaken. A person skilled in the art would recognize that the vimentin gene is a human gene and that such assays would evaluate the amount of its methylation. Ex. 1014 at paragraph 172.

Ahlquist in view of De Luca and Olson therefore discloses the additional limitations of claim 6 of the ‘781 Patent. Because the other limitations of claim 6 are obvious for the reasons explained above for claim 5, claim 6 is also obvious over Ahlquist in view of De Luca and Olson.

4. O’Neill in view of De Luca, Olson, and Leung Renders Claim 6 Obvious

‘781 Patent, Claim 6	O’Neill in view of De Luca, Olson, and Leung
6. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises measuring an amount of a methylated human DNA.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of a methylated human DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of a methylated human DNA in feces as disclosed in claim 6. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to measure an amount of a methylated human DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 6. Because the other limitations of claim 6 are obvious for the reasons explained above for claim 5, claim 6 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

G. Dependent Claim 7

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 7 Obvious

'781 Patent, Claim 7	Lapidus '178 in view of De Luca, Olson, and Leung
7. The method of claim 5, wherein the epigenetic modification comprises aberrant methylation.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Lapidus '178 in view of De Luca, Olson, and Leung disclose the limitations of claim 6. Leung also discloses evaluation of human DNA from feces, including the detection of aberrantly methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect aberrantly methylated DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect aberrantly methylated DNA in feces as disclosed in claim 7. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a

reasonable expectation of success to detect aberrantly methylated DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 7. Because the other limitations of claim 7 are obvious for the reasons explained above for claim 5, claim 7 is also obvious over Lapidus '178 in view of De Luca, Olson, and Leung.

2. Lapidus '650 in view of De Luca, Olson, and Leung Renders Claim 7 Obvious

'781 Patent, Claim 7	Lapidus '650 in view of De Luca, Olson, and Leung
7. The method of claim 5, wherein the epigenetic modification comprises aberrant methylation.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Lapidus '650 in view of De Luca, Olson, and Leung disclose the limitations of claim 6. Leung also discloses evaluation of human DNA from feces, including the detection of aberrantly methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect aberrantly methylated DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect aberrantly methylated DNA in feces as disclosed in claim 7. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect aberrantly methylated DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 7. Because the other limitations of claim 7 are obvious for the reasons explained above for claim 5, claim 7 is also obvious over Lapidus '650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 7 Obvious

'781 Patent, Claim 7	Ahlquist in view of De Luca, Olson, and Leung
7. The method of claim 5, wherein the epigenetic modification comprises aberrant methylation.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 6. As discussed *supra*, Ahlquist discloses determining expression from a human gene, wherein the nucleic acid is tested for the presence of human DNA having an epigenetic modification. However, Ahlquist/De Luca/Olson does not specifically disclose testing for aberrant methylation.

Leung also discloses evaluation of human DNA from feces, including the detection of aberrantly methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect aberrantly methylated DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect aberrantly methylated DNA in feces as disclosed in claim 7. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect aberrantly methylated DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 7. Because the other limitations of claim 7 are obvious for the reasons explained above for claim 5, claim 7 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 7 Obvious

'781 Patent, Claim 7	O'Neill in view of De Luca, Olson, and Leung
7. The method of claim 5, wherein the epigenetic modification comprises aberrant methylation.	Leung discloses “detecting aberrantly methylated DNA ... in feces of patients with colorectal cancer or polyps.” Ex. 1008 at p. 1070, Abstract (Background).

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 6. Leung also discloses evaluation of human DNA from feces, including the detection of aberrantly methylated DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect aberrantly methylated DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect aberrantly methylated DNA in feces as disclosed in claim 7. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect aberrantly methylated DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 7. Because the other limitations of claim 7 are obvious for the reasons explained above for claim 5, claim 7 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

H. Dependent Claim 8

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 8 Obvious

'781 Patent, Claim 8	Lapidus '178 in view of De Luca, Olson, and Leung
8. The method of claim 7, wherein the aberrant methylation comprises hypermethylation.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients

	with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).
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As discussed *supra*, Lapidus ‘178 in view of De Luca, Olson, and Leung disclose the limitations of claim 7. Further, Leung also discloses evaluation of human DNA from feces, including the detection of hypermethylation of DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect hypermethylation of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect hypermethylation of DNA in feces as disclosed in claim 8. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect hypermethylation of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 8. Because the other limitations of claim 8 are obvious for the reasons explained above for claim 7, claim 8 is also obvious over Lapidus ‘178 in view of De Luca, Olson, and Leung.

2. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 8 Obvious

‘781 Patent, Claim 8	Lapidus ‘650 in view of De Luca, Olson, and Leung
8. The method of claim 7, wherein the aberrant methylation comprises hypermethylation.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of

	hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).
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As discussed *supra*, Lapidus ‘650 in view of De Luca, Olson, and Leung disclose the limitations of claim 7. Further, Leung also discloses evaluation of human DNA from feces, including the detection of hypermethylation of DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect hypermethylation of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect hypermethylation of DNA in feces as disclosed in claim 8. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect hypermethylation of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 8. Because the other limitations of claim 8 are obvious for the reasons explained above for claim 7, claim 8 is also obvious over Lapidus ‘650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 8 Obvious

‘781 Patent, Claim 8	Ahlquist in view of De Luca, Olson, and Leung
8. The method of claim 7, wherein the aberrant methylation comprises hypermethylation.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF,

	MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).
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As discussed *supra*, Ahlquist in view of De Luca, Olson, and Leung disclose the limitations of claim 7. Further, Leung also discloses evaluation of human DNA from feces, including the detection of hypermethylation of DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect hypermethylation of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect hypermethylation of DNA in feces as disclosed in claim 8. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect hypermethylation of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 8. Because the other limitations of claim 8 are obvious for the reasons explained above for claim 7, claim 8 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 8 Obvious

'781 Patent, Claim 8	O'Neill in view of De Luca, Olson, and Leung
8. The method of claim 7, wherein the aberrant methylation comprises hypermethylation.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 7. Further, Leung also discloses evaluation of human DNA from feces, including the detection of hypermethylation of DNA for utilization in colorectal cancer evaluations of the patients. Thus, Leung teaches that before the time of filing, it was routine in the art to detect hypermethylation of DNA in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect hypermethylation of DNA in feces as disclosed in claim 8. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect hypermethylation of DNA because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 8. Because the other limitations of claim 8 are obvious for the reasons explained above for claim 7, claim 8 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

I. Dependent Claim 9

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 9 Obvious

'781 Patent, Claim 9	Lapidus '178 in view of De Luca, Olson, and Leung
9. The method of claim 5, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).

As discussed *supra*, Lapidus '178 in view of De Luca, Olson, and Leung disclose the limitations of claim 5.

The '781 patent defines "epigenetic modification" such that it includes "any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene." '781 patent at column 12, lines 35-39. Furthermore, the '781 patent states that "[i]n specific embodiments, the epigenetic modification is methylation." *Id.* at column 12, line 46-47.

As discussed *supra*, Lapidus '178 and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, Lapidus '178/De Luca/Olson does not specifically disclose a human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. For instance, Leung teaches analysis in stool by methylation-specific PCR for the presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). Thus, Leung teaches that before the time of filing, it was routine in the art to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene as disclosed in claim 5. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene because Leung teaches that this evaluation is routine in the art. *Id.*

Lapidus '178 in view of De Luca, Olson, and Leung therefore discloses the additional limitations of claim 9 of the '781 Patent. Because the other limitations of claim 9 are obvious for the reasons explained above for claim 5, claim 9 is also obvious over Lapidus '178 in view of De Luca, Olson, and Leung.

2. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 9 Obvious

‘781 Patent, Claim 9	Lapidus ‘650 in view of De Luca, Olson, and Leung
9. The method of claim 5, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).

As discussed *supra*, Lapidus ‘650 in view of De Luca, Olson, and Leung disclose the limitations of claim 5.

The ‘781 patent defines “epigenetic modification” such that it includes “any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene.” ‘781 patent at column 12, lines 35-39. Furthermore, the ‘781 patent states that “[i]n specific embodiments, the epigenetic modification is methylation.” *Id.* at column 12, line 46-47.

As discussed *supra*, Lapidus ‘650 and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, Lapidus ‘650/De Luca/Olson does not specifically disclose a human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. For instance, Leung teaches analysis in stool by methylation-specific PCR for the presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1).

Thus, Leung teaches that before the time of filing, it was routine in the art to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene as disclosed in claim 5. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene because Leung teaches that this evaluation is routine in the art. *Id.*

Lapidus '650 in view of De Luca, Olson, and Leung therefore discloses the additional limitations of claim 9 of the '781 Patent. Because the other limitations of claim 9 are obvious for the reasons explained above for claim 5, claim 9 is also obvious over Lapidus '650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca and Olson Renders Claim 9 Obvious

'781 Patent, Claim 9	Ahlquist in view of De Luca and Olson
9. The method of claim 5, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.	Ahlquist discloses for evaluation of the second stool DNA test (SDT-1), “sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): K-ras mutations, scanning of APC mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant Kras (12), APC scanning (25), and vimentin gene methylation (20) assays.” Ex. 1004, page 4 at ‘DNA Testing’ heading.

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 5.

The '781 patent defines "epigenetic modification" such that it includes "any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene." '781 patent at column 12, lines 35-39. Furthermore, the '781 patent states that "[i]n specific embodiments, the epigenetic modification is methylation." *Id.* at column 12, line 46-47.

Ahlquist teaches determining expression from a human gene, wherein the nucleic acid is tested for the presence of human DNA having an epigenetic modification. In particular, Ahlquist discloses that assays for "methylation of the vimentin gene" were undertaken. A person skilled in the art would recognize that the vimentin gene is a human gene and that evaluation of its methylation would meet the definition of testing for an epigenetic modification comprises a gene. Ex. 1014 at paragraph 172.

Ahlquist in view of De Luca and Olson therefore discloses the additional limitations of claim 9 of the '781 Patent. Because the other limitations of claim 9 are obvious for the reasons explained above for claim 5, claim 9 is also obvious over Ahlquist in view of De Luca and Olson.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 9 Obvious

'781 Patent, Claim 9	O'Neill in view of De Luca, Olson, and Leung
9. The method of claim 5, wherein the human DNA having an epigenetic modification comprises a gene and/or a promoter region of a gene.	Leung discloses that "[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at p. 1070, Abstract (Methods).

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 5.

The '781 patent defines "epigenetic modification" such that it includes "any alteration in the DNA, generally resulting in diminished gene expression, which is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene." '781 patent at column 12, lines 35-39. Furthermore, the '781 patent states that "[i]n specific embodiments, the epigenetic modification is methylation." *Id.* at column 12, line 46-47.

As discussed *supra*, O'Neill and Olson discloses testing DNA and/or RNA from portions of a fecal sample. However, O'Neill/De Luca/Olson does not specifically disclose a human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene.

Leung also discloses evaluation of human DNA from feces, including the detection of methylated DNA for utilization in colorectal cancer evaluations of the patients. For instance, Leung teaches analysis in stool by methylation-specific PCR for the presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). Thus, Leung teaches that before the time of filing, it was routine in the art to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene as disclosed in claim 5. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect human DNA having an epigenetic modification that comprises a gene and/or a promoter region of a gene because Leung teaches that this evaluation is routine in the art. *Id.*

O'Neill in view of De Luca, Olson, and Leung therefore discloses the additional limitations of claim 9 of the '781 Patent. Because the other limitations of claim 9 are obvious for the reasons explained above for claim 5, claim 9 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

J. Dependent Claim 10

1. Lapidus ‘178 in view of De Luca, Olson, and Leung Renders Claim 10 Obvious

‘781 Patent, Claim 10	Lapidus ‘178 in view of De Luca, Olson, and Leung
10. The method of claim 9, wherein the gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFP12, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at page 1070, Abstract (Methods).

As discussed *supra*, Lapidus ‘178 in view of De Luca, Olson, and Leung disclose the limitations of claim 9. Leung also discloses evaluation of human DNA from feces, including the detection of an epigenetic modification (hypermethylated DNA) in at least 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). A person of ordinary skill in the art would understand that ‘sFRP2’ as taught by Leung and ‘SFRP2’ as stated in claim 10 are the same gene. Thus, Leung teaches that before the time of filing, it was routine in the art to detect epigenetic modification of the SFRP2 gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect epigenetic modification of the SFRP2 gene in feces as disclosed in claim 10. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect epigenetic modification of the SFRP2 gene because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 10. Because the other limitations of claim 10 are obvious for the reasons explained above for claim 9, claim 10 is also obvious over Lapidus ‘178 in view of De Luca, Olson, and Leung.

2. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 10 Obvious

‘781 Patent, Claim 10	Lapidus ‘650 in view of De Luca, Olson, and Leung
10. The method of claim 9, wherein the gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFP12, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at page 1070, Abstract (Methods).

As discussed *supra*, Lapidus ‘650 in view of De Luca, Olson, and Leung disclose the limitations of claim 9. Leung also discloses evaluation of human DNA from feces, including the detection of an epigenetic modification (hypermethylated DNA) in at least 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). A person of ordinary skill in the art would understand that ‘sFRP2’ as taught by Leung and ‘SFRP2’ as stated in claim 10 are the same gene. Thus, Leung teaches that before the time of filing, it was routine in the art to detect epigenetic modification of the SFRP2 gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect epigenetic modification of the SFRP2 gene in feces as disclosed in claim 10. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect epigenetic modification of the SFRP2 gene because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 10. Because the other limitations of claim 10 are obvious for the reasons explained above for claim 9, claim 10 is also obvious over Lapidus ‘650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 10 Obvious

‘781 Patent, Claim 10	Ahlquist in view of De Luca, Olson, and Leung
10. The method of claim 9, wherein the gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFP12, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at page 1070, Abstract (Methods).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 9. As discussed *supra*, Ahlquist discloses determining expression from the human gene by testing for the presence of human DNA having an epigenetic modification. However, Ahlquist/De Luca/Olson does not specifically disclose testing for a gene listed in claim 10.

Leung also discloses evaluation of human DNA from feces, including the detection of an epigenetic modification (hypermethylated DNA) in at least 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). A person of ordinary skill in the art would understand that ‘sFRP2’ as taught by Leung and ‘SFRP2’ as stated in claim 10 are the same gene. Thus, Leung teaches that before the time of filing, it was routine in the art to detect epigenetic modification of the SFRP2 gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect epigenetic modification of the SFRP2 gene in feces as disclosed in claim 10. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect epigenetic modification of the SFRP2 gene because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 10. Because the other limitations of claim 10 are obvious for the reasons explained above for claim 9, claim 10 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 10 Obvious

'781 Patent, Claim 10	O'Neill in view of De Luca, Olson, and Leung
10. The method of claim 9, wherein the gene is selected from the group consisting of PHACTR3, NDRG4, FOXE1, GATA4, GPNMB, TFP12, SOX17, SYNE1, LAMA, MMP2, OSMR, SFRP2, and CDO1.	Leung discloses that “[f]ecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1) in stool was analyzed by methylation-specific PCR. Ex. 1008 at page 1070, Abstract (Methods).

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 9. Leung also discloses evaluation of human DNA from feces, including the detection of an epigenetic modification (hypermethylated DNA) in at least 7 tumor-related genes (APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1). A person of ordinary skill in the art would understand that 'sFRP2' as taught by Leung and 'SFRP2' as stated in claim 10 are the same gene. Thus, Leung teaches that before the time of filing, it was routine in the art to detect epigenetic modification of the SFRP2 gene in feces. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to detect epigenetic modification of the SFRP2 gene in feces as disclosed in claim 10. Ex. 1014 at paragraphs 171 and 173. A skilled person would have a reasonable expectation of success to detect epigenetic modification of the SFRP2 gene because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 10. Because the other limitations of claim 10 are obvious for the reasons explained above for claim 9, claim 10 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

K. Dependent Claim 11

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 11 Obvious

'781 Patent, Claim 11	Lapidus '178 in view of De Luca, Olson, and Leung
11. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.	Leung discloses that “[t]he presence of methylated DNA in stool was detected by methylation-specific PCR (MSP). Briefly, 2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil by the EZ DNA methylation kit (Zymo Research, Orange, CA). Ex. 1008 at page 1071, second column, first full paragraph.

As discussed *supra*, Lapidus '178 in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces. Further, Leung teaches that methylated DNA in stool was detected by methylation-specific PCR that utilized the EZ DNA methylation kit from Zymo Research in which “2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil.”

It is well known in the art that the EZ DNA methylation kit from Zymo Research utilizes bisulfate ions for modifying the nucleic acid with under conditions wherein unmethylated cytosine is converted to uracil. Ex. 1014 at paragraphs 171 and 173. Thus, Leung teaches that before the time of filing, it was routine in the art to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications as

disclosed in claim 11. *Id.* A skilled person would have a reasonable expectation of success to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 11. Because the other limitations of claim 11 are obvious for the reasons explained above for claim 5, claim 11 is also obvious over Lapidus '178 in view of De Luca, Olson, and Leung.

2. Lapidus '650 in view of De Luca, Olson, and Leung Renders Claim 11 Obvious

'781 Patent, Claim 11	Lapidus '650 in view of De Luca, Olson, and Leung
11. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.	Leung discloses that “[t]he presence of methylated DNA in stool was detected by methylation-specific PCR (MSP). Briefly, 2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil by the EZ DNA methylation kit (Zymo Research, Orange, CA). Ex. 1008 at page 1071, second column, first full paragraph.

As discussed *supra*, Lapidus '650 in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces. Further, Leung teaches that methylated DNA in stool was detected by methylation-specific PCR that utilized the EZ DNA methylation kit from Zymo Research in which “2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil.”

It is well known in the art that the EZ DNA methylation kit from Zymo Research utilizes bisulfate ions for modifying the nucleic acid with under conditions wherein unmethylated cytosine is converted to uracil. Ex. 1014 at paragraphs 171 and 173. Thus, Leung teaches that before the time of filing, it was routine in the art to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic

modifications. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications as disclosed in claim 11. *Id.* A skilled person would have a reasonable expectation of success to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 11. Because the other limitations of claim 11 are obvious for the reasons explained above for claim 5, claim 11 is also obvious over Lapidus '650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 11 Obvious

'781 Patent, Claim 11	Ahlquist in view of De Luca, Olson, and Leung
11. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.	Leung discloses that “[t]he presence of methylated DNA in stool was detected by methylation-specific PCR (MSP). Briefly, 2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil by the EZ DNA methylation kit (Zymo Research, Orange, CA). Ex. 1008 at page 1071, second column, first full paragraph.

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 5. As discussed *supra*, Ahlquist discloses determining expression from the human gene by testing for the presence of human DNA having an epigenetic modification. However, Ahlquist/De Luca/Olson does not specifically disclose modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.

Leung also discloses evaluation of human DNA from feces. Further, Leung teaches that methylated DNA in stool was detected by methylation-specific PCR that utilized the EZ DNA

methylation kit from Zymo Research in which “2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil.”

It is well known in the art that the EZ DNA methylation kit from Zymo Research utilizes bisulfate ions for modifying the nucleic acid with under conditions wherein unmethylated cytosine is converted to uracil. Ex. 1014 at paragraphs 171 and 173. Thus, Leung teaches that before the time of filing, it was routine in the art to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications as disclosed in claim 11. *Id.* A skilled person would have a reasonable expectation of success to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 11. Because the other limitations of claim 11 are obvious for the reasons explained above for claim 5, claim 11 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

4. O'Neill in view of De Luca, Olson, and Leung Renders Claim 11 Obvious

'781 Patent, Claim 11	O'Neill in view of De Luca, Olson, and Leung
11. The method of claim 5, wherein testing the nucleic acid for the presence of human DNA having an epigenetic modification comprises modifying the nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil.	Leung discloses that “[t]he presence of methylated DNA in stool was detected by methylation-specific PCR (MSP). Briefly, 2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil by the EZ DNA methylation kit (Zymo Research, Orange, CA). Ex. 1008 at page 1071, second column, first full paragraph.

As discussed *supra*, O'Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 5. Leung also discloses evaluation of human DNA from feces. Further, Leung teaches that methylated DNA in stool was detected by methylation-specific PCR that utilized the EZ DNA methylation kit from Zymo Research in which "2 µg of DNA was chemically modified to convert all unmethylated cytosine to uracil."

It is well known in the art that the EZ DNA methylation kit from Zymo Research utilizes bisulfate ions for modifying the nucleic acid with under conditions wherein unmethylated cytosine is converted to uracil. Ex. 1014 at paragraphs 171 and 173. Thus, Leung teaches that before the time of filing, it was routine in the art to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications as disclosed in claim 11. *Id.* A skilled person would have a reasonable expectation of success to modify nucleic acid with bisulfate ions under conditions wherein unmethylated cytosine is converted to uracil in order to test for epigenetic modifications because Leung teaches that this evaluation is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 11. Because the other limitations of claim 11 are obvious for the reasons explained above for claim 5, claim 11 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

L. Dependent Claim 12

1. Lapidus '178 Anticipates Claim 12

'781 Patent, Claim 12	Lapidus '178
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus '178 describes "DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA." Ex. 1002 at column 9, lines 47-49. Furthermore, Lapidus '178 describes that "[n]on-limiting examples of useful known

	assay protocols include those disclosed in U.S. Pat. Nos. 5,137,806 (detection of sequences in selected DNA molecules), 5,348,855 (assay for nucleic acid sequences), 5,512,441 (detection of mutant alleles), 5,272,057 and 5,380,645 (RFLP analysis), 5,527,676 (detection of p53 gene sequences), 5,330,892 (detection of MCC gene sequences), 5,352,775 (detection of APC gene sequences), 5,532,108 (detection of DCC gene sequences), and in WO96/08514 (monoclonal antibodies against human colon carcinoma-associated antigens), the disclosures of each of which are incorporated by reference herein.” <i>Id.</i> at column 6, line 58 – column 7, line 2.
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Lapidus ‘178 describes determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus ‘178 describes that RNA can be isolated according to methods known in the art and that testing may be performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations.

As discussed *supra*, Lapidus ‘178 expressly incorporates by reference dozens of assays on nucleic acids, including those described in U.S. Pat. Nos. 5,137,806, 5,348,855, 5,512,441, 5,272,057, 5,380,645, 5,527,676, 5,330,892, 5,352,775, and 5,532,108. For instance, U.S. Patent No. 5,527,676 (Ex. 1013; incorporated by reference into Lapidus ‘178 at column 6, lines 63-64) provides a teaching that relatively little expression of p53 mRNA was observed compared to other tumors. This low level of expression of p53 was specific in that c-myc, histone H3, and phosphoglycerate kinase mRNAs were expressed in these four tumors at levels similar to those seen in other colorectal tumors and at least as high as in non-neoplastic colonic mucosa. A

person of ordinary skill in the art understands that such assay involves determining expression from a human gene by measuring an amount of RNA expressed from the gene extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraph 179. Thus, Lapidus '178 describes assays for determining expression from human gene comprising measuring an amount of RNA expressed from the gene.

Lapidus '178 therefore describes the additional limitations of claim 12 of the '781 Patent. Because the other limitations of claim 12 are anticipated for the reasons explained above for claims 1, 3, and 4, claim 12 is also anticipated by Lapidus '178.

2. Lapidus '650 Anticipates Claim 12

'781 Patent, Claim 12	Lapidus '650
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus '650 describes "DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA." Ex. 1003 at column 8, lines 22-25. Furthermore, Lapidus '650 describes that "[n]on-limiting examples of useful known assay protocols include those disclosed in U.S. Pat. No. 5,137,806 (detection of sequences in selected DNA molecules), U.S. Pat. No. 5,348,855 (assay for nucleic acid sequences), U.S. Pat. No. 5,512,441 (detection of mutant alleles), U.S. Pat. No. 5,272,057 and U.S. Pat. No. 5,380,645 (RFLP analysis), U.S. Pat. No. 5,527,676 (detection of p53 gene sequences), U.S. Pat. No. 5,330,892 (detection of MCC gene sequences), U.S. Pat. No. 5,352,775 (detection of APC gene sequences), U.S. Pat. No. 5,532,108 (detection of DCC gene

	sequences), and in WO96/08514 (monoclonal antibodies against human colon carcinoma-associated antigens), the disclosures of each of which are incorporated by reference herein.” <i>Id.</i> at column 6, lines 15-28.
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Lapidus ‘650 describes determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus ‘650 describes that RNA can be isolated according to methods known in the art and that testing may be performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations.

As discussed *supra*, Lapidus ‘650 expressly incorporates by reference dozens of assays on nucleic acids, including those described in U.S. Pat. Nos. 5,137,806, 5,348,855, 5,512,441, 5,272,057, 5,380,645, 5,527,676, 5,330,892, 5,352,775, and 5,532,108. For instance, U.S. Patent No. 5,527,676 (Ex. 1013; incorporated by reference into Lapidus ‘650 at column 6, lines 15-26) provides a teaching that relatively little expression of p53 mRNA was observed compared to other tumors. This low level of expression of p53 was specific in that c-myc, histone H3, and phosphoglycerate kinase mRNAs were expressed in these four tumors at levels similar to those seen in other colorectal tumors and at least as high as in non-neoplastic colonic mucosa. A person of ordinary skill in the art understands that such assay involves determining expression from a human gene by measuring an amount of RNA expressed from the gene extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraph 179. Thus, Lapidus ‘650 describes assays for determining expression from human gene comprising measuring an amount of RNA expressed from the gene.

Lapidus ‘650 therefore describes the additional limitations of claim 12 of the ‘781 Patent. Because the other limitations of claim 12 are anticipated for the reasons explained above for claims 1, 3, and 4, claim 12 is also anticipated by Lapidus ‘650.

3. Lapidus '178 Renders Claim 12 Obvious

'781 Patent, Claim 12	Lapidus '178
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus '178 discloses “DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA.” Ex. 1002 at column 9, lines 47-49. Furthermore, Lapidus '178 discloses that “[n]on-limiting examples of useful known assay protocols include those disclosed in U.S. Pat. Nos. 5,137,806 (detection of sequences in selected DNA molecules), 5,348,855 (assay for nucleic acid sequences), 5,512,441 (detection of mutant alleles), 5,272,057 and 5,380,645 (RFLP analysis), 5,527,676 (detection of p53 gene sequences), 5,330,892 (detection of MCC gene sequences), 5,352,775 (detection of APC gene sequences), 5,532,108 (detection of DCC gene sequences), and in WO96/08514 (monoclonal antibodies against human colon carcinoma-associated antigens), the disclosures of each of which are incorporated by reference herein.” <i>Id.</i> at column 6, line 58 – column 7, line 2.

In the alternative to the assertion that Lapidus '178 anticipates claim 12, Lapidus '178 further renders claim 12 obvious.

Lapidus '178 discloses determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus '178 discloses that RNA can be isolated according to methods known in the art and that testing may be

performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations. Ex. 1014 at paragraphs 179-180.

As discussed *supra*, Lapidus '178 expressly incorporates by reference dozens of assays on nucleic acids, including those described in U.S. Pat. Nos. 5,137,806, 5,348,855, 5,512,441, 5,272,057, 5,380,645, 5,527,676, 5,330,892, 5,352,775, and 5,532,108. For instance, U.S. Patent No. 5,527,676 (Ex. 1013; incorporated by reference into Lapidus '178 at column 6, lines 63-64) provides a teaching that relatively little expression of p53 mRNA was observed compared to other tumors. This low level of expression of p53 was specific in that c-myc, histone H3, and phosphoglycerate kinase mRNAs were expressed in these four tumors at levels similar to those seen in other colorectal tumors and at least as high as in non-neoplastic colonic mucosa. A person of ordinary skill in the art understands that such assay involves determining expression from a human gene by measuring an amount of RNA expressed from the gene extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraph 179. Thus, Lapidus '178 discloses assays for determining expression from human gene comprising measuring an amount of RNA expressed from the gene.

Lapidus '178 therefore describes the additional limitations of claim 12 of the '781 Patent. Because the other limitations of claim 12 are obvious for the reasons explained above for claims 1, 3, and 4, claim 12 is obvious over Lapidus '178.

4. Lapidus '650 Renders Claim 12 Obvious

'781 Patent, Claim 12	Lapidus '650
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus '650 describes "DNA or RNA may be isolated from the homogenate using methods known in the art. Subsequent tests may be performed on the isolated DNA and RNA." Ex. 1003 at column 8, lines 22-25. Furthermore, Lapidus '650 describes that "[n]on-limiting examples of useful known assay protocols include those disclosed in

	U.S. Pat. No. 5,137,806 (detection of sequences in selected DNA molecules), U.S. Pat. No. 5,348,855 (assay for nucleic acid sequences), U.S. Pat. No. 5,512,441 (detection of mutant alleles), U.S. Pat. No. 5,272,057 and U.S. Pat. No. 5,380,645 (RFLP analysis), U.S. Pat. No. 5,527,676 (detection of p53 gene sequences), U.S. Pat. No. 5,330,892 (detection of MCC gene sequences), U.S. Pat. No. 5,352,775 (detection of APC gene sequences), U.S. Pat. No. 5,532,108 (detection of DCC gene sequences), and in WO96/08514 (monoclonal antibodies against human colon carcinoma-associated antigens), the disclosures of each of which are incorporated by reference herein.” <i>Id.</i> at column 6, lines 15-28.
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In the alternative to the assertion that Lapidus ‘650 anticipates claim 12, Lapidus ‘650 further renders claim 12 obvious.

Lapidus ‘650 discloses determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus ‘650 discloses that RNA can be isolated according to methods known in the art and that testing may be performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations. Ex. 1014 at paragraphs 179-180.

As discussed *supra*, Lapidus ‘650 expressly incorporates by reference dozens of assays on nucleic acids, including those described in U.S. Pat. Nos. 5,137,806, 5,348,855, 5,512,441, 5,272,057, 5,380,645, 5,527,676, 5,330,892, 5,352,775, and 5,532,108. For instance, U.S. Patent No. 5,527,676 (Ex. 1013; incorporated by reference into Lapidus ‘650 at column 6, lines 15-26) provides a teaching that relatively little expression of p53 mRNA was observed compared to

other tumors. This low level of expression of p53 was specific in that c-myc, histone H3, and phosphoglycerate kinase mRNAs were expressed in these four tumors at levels similar to those seen in other colorectal tumors and at least as high as in non-neoplastic colonic mucosa. A person of ordinary skill in the art understands that such assay involves determining expression from a human gene by measuring an amount of RNA expressed from the gene extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraph 179. Thus, Lapidus '650 discloses assays for determining expression from human gene comprising measuring an amount of RNA expressed from the gene.

Lapidus '650 therefore describes the additional limitations of claim 12 of the '781 Patent. Because the other limitations of claim 12 are obvious for the reasons explained above for claims 1, 3, and 4, claim 12 is obvious over Lapidus '650.

5. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 12 Obvious

'781 Patent, Claim 12	Lapidus '178 in view of De Luca, Olson, and Leung
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus '178 discloses “[m]ethods of the invention are used to prepare a stool sample followed by detection of a deletion or other mutation in the p53 tumor suppressor gene. ... DNA or RNA may optionally be isolated from the sample according to methods known in the art.” Ex. 1002 at column 9, line 62 – column 10, line 6. Leung discloses that “COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit

	(Qiagen).” Ex. 1008 at page 1071, second column, second full paragraph.
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 4. As discussed *supra*, Lapidus ‘178 and Olson discloses testing human DNA and/or RNA from portions of a fecal sample. Lapidus ‘178 discloses determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus ‘178 discloses that RNA can be isolated according to methods known in the art and that testing may be performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations. Ex. 1014 at paragraph 179.

Even if Lapidus ‘178 is determined to not specifically disclose determining expression from the human gene comprises measuring an amount of RNA expressed from the gene, Leung removes this deficiency.

Leung also discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2). Thus, Leung teaches that before the time of filing, it was routine in the art to test RNA from stool to determine expression from the COX-2 gene. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene as disclosed in claim 12. Ex. 1014 at paragraph 181. A skilled person would have a reasonable expectation of success to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 12. Because the other limitations of claim 12 are obvious for the reasons explained above for claim 4, claim 12 is also obvious over Lapidus ‘178 in view of De Luca, Olson, and Leung.

6. Lapidus ‘650 in view of De Luca, Olson, and Leung Renders Claim 12 Obvious

‘781 Patent, Claim 12	Lapidus ‘650 in view of De Luca, Olson, and Leung
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Lapidus ‘650 discloses “[m]ethods of the invention are used to prepare a stool sample followed by detection of a deletion or other mutation in the p53 tumor suppressor gene.” ... DNA or RNA may optionally be isolated from the sample according to methods known in the art.” <i>Id.</i> at column 8, lines 39-50. Leung discloses that “COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen).” Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 4. As discussed *supra*, Lapidus ‘650 and Olson discloses testing human DNA and/or RNA from portions of a fecal sample. Lapidus ‘650 discloses determining expression from the human gene comprises measuring an amount of RNA expressed from the gene. In particular, Lapidus ‘650 discloses that RNA can be isolated according to methods known in the art and that testing may be performed on the isolated RNA. One of ordinary skill in the art would understand that RNA expression from a particular gene, especially one utilized for the possible detection of colon cancer, could be an assay included in such evaluations. Ex. 1014 at paragraph 179.

Even if Lapidus ‘650 is determined to not specifically disclose determining expression from the human gene comprises measuring an amount of RNA expressed from the gene, Leung removes this deficiency.

Leung also discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2). Thus, Leung teaches that before the time of filing, it was routine in the art to test RNA from stool to determine expression from the COX-2 gene. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene as disclosed in claim 12. Ex. 1014 at paragraph 181. A skilled person would have a reasonable expectation of success to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 12. Because the other limitations of claim 12 are obvious for the reasons explained above for claim 4, claim 12 is also obvious over Lapidus '650 in view of De Luca, Olson, and Leung.

7. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 12 Obvious

'781 Patent, Claim 12	Ahlquist in view of De Luca, Olson, and Leung
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Leung discloses that "COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen)." Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 4. As discussed *supra*, Ahlquist discloses testing the nucleic acid comprises determining expression from a human gene. However, Ahlquist/De Luca/Olson does not specifically disclose determining expression from the human gene comprising measuring an amount of RNA expressed from the gene.

Leung also discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2). Thus,

Leung teaches that before the time of filing, it was routine in the art to test RNA from stool to determine expression from the COX-2 gene. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene as disclosed in claim 12. Ex. 1014 at paragraph 181. A skilled person would have a reasonable expectation of success to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 12. Because the other limitations of claim 12 are obvious for the reasons explained above for claim 4, claim 12 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

8. O'Neill in view of De Luca, Olson, and Leung Renders Claim 12 Obvious

'781 Patent, Claim 12	O'Neill in view of De Luca, Olson, and Leung
12. The method of claim 4, wherein determining expression from the human gene comprises measuring an amount of RNA expressed from the gene.	Leung discloses that "COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen)." Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 4. As discussed *supra*, O'Neill and Olson discloses testing human DNA and/or RNA from portions of a fecal sample. However, O'Neill/De Luca/Olson does not specifically disclose determining expression from the human gene comprising measuring an amount of RNA expressed from the gene.

Leung also discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2). Thus, Leung teaches that before the time of filing, it was routine in the art to test RNA from stool to determine expression from the COX-2 gene. As such, a person of ordinary skill in the art would

be motivated to utilize the teachings of Leung to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene as disclosed in claim 12. Ex. 1014 at paragraph 181. A skilled person would have a reasonable expectation of success to determine expression from the human gene comprises measuring an amount of RNA expressed from the gene because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 12. Because the other limitations of claim 12 are obvious for the reasons explained above for claim 4, claim 12 is also obvious over O'Neill in view of De Luca, Olson, and Leung.

M. Dependent Claim 13

1. Lapidus '178 in view of De Luca, Olson, and Leung Renders Claim 13 Obvious

'781 Patent, Claim 13	Lapidus '178 in view of De Luca, Olson, and Leung
13. The method of claim 12, wherein measuring an amount of RNA expressed from the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).	Leung discloses that "COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen)." Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, Lapidus '178 in view of De Luca, Olson, and Leung disclose the limitations of claim 12. Leung discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2) via reverse transcriptase polymerase chain reaction (RT-PCR). Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of RNA expressed from a gene using RT-PCR. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of RNA expressed from a gene using RT-PCR as disclosed in claim 13. Ex. 1014 at paragraph 191. A skilled person would have a reasonable expectation of success to measure an amount of RNA expressed from a gene using RT-PCR because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 13. Because the other limitations of claim 13 are obvious for the reasons explained above for claim 12, claim 13 is also obvious over Lapidus '178 in view of De Luca, Olson, and Leung.

2. Lapidus '650 in view of De Luca, Olson, and Leung Renders Claim 13 Obvious

'781 Patent, Claim 13	Lapidus '650 in view of De Luca, Olson, and Leung
13. The method of claim 12, wherein measuring an amount of RNA expressed from the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).	Leung discloses that "COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen)." Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, Lapidus '650 in view of De Luca, Olson, and Leung disclose the limitations of claim 12. Leung discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2) via reverse transcriptase polymerase chain reaction (RT-PCR). Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of RNA expressed from a gene using RT-PCR. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of RNA expressed from a gene using RT-PCR as disclosed in claim 13. Ex. 1014 at paragraph 191. A skilled person would have a reasonable expectation of success to measure an amount of RNA expressed from a gene using RT-PCR because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 13. Because the other limitations of claim 13 are obvious for the reasons explained above for claim 12, claim 13 is also obvious over Lapidus '650 in view of De Luca, Olson, and Leung.

3. Ahlquist in view of De Luca, Olson, and Leung Renders Claim 13 Obvious

‘781 Patent, Claim 13	Ahlquist in view of De Luca, Olson, and Leung
13. The method of claim 12, wherein measuring an amount of RNA expressed from the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).	Leung discloses that “COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen).” Ex. 1008 at page 1071, second column, second full paragraph.

As discussed *supra*, Ahlquist in view of De Luca, Olson, and Leung disclose the limitations of claim 12. Leung discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2) via reverse transcriptase polymerase chain reaction (RT-PCR). Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of RNA expressed from a gene using RT-PCR. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of RNA expressed from a gene using RT-PCR as disclosed in claim 13. Ex. 1014 at paragraph 191. A skilled person would have a reasonable expectation of success to measure an amount of RNA expressed from a gene using RT-PCR because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 13. Because the other limitations of claim 13 are obvious for the reasons explained above for claim 12, claim 13 is also obvious over Ahlquist in view of De Luca, Olson, and Leung.

4. O’Neill in view of De Luca, Olson, and Leung Renders Claim 13 Obvious

‘781 Patent, Claim 13	O’Neill in view of De Luca, Olson, and Leung
13. The method of claim 12, wherein measuring an amount of RNA expressed from	Leung discloses that “COX-2 expression in stool samples was detected by RT-PCR.

the gene comprises reverse transcriptase polymerase chain reaction (RT-PCR).	Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen).” Ex. 1008 at page 1071, second column, second full paragraph.
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As discussed *supra*, O’Neill in view of De Luca, Olson, and Leung disclose the limitations of claim 12. Leung discloses evaluation of nucleic acid from feces. Further, Leung teaches that mRNA was evaluated from stool to determine expression of cyclooxygenase-2 (COX-2) via reverse transcriptase polymerase chain reaction (RT-PCR). Thus, Leung teaches that before the time of filing, it was routine in the art to measure an amount of RNA expressed from a gene using RT-PCR. As such, a person of ordinary skill in the art would be motivated to utilize the teachings of Leung to measure an amount of RNA expressed from a gene using RT-PCR as disclosed in claim 13. Ex. 1014 at paragraph 191. A skilled person would have a reasonable expectation of success to measure an amount of RNA expressed from a gene using RT-PCR because Leung teaches that this testing is routine in the art. *Id.*

Leung therefore teaches the additional limitation of claim 13. Because the other limitations of claim 13 are obvious for the reasons explained above for claim 12, claim 13 is also obvious over O’Neill in view of De Luca, Olson, and Leung.

N. Dependent Claim 14

1. Lapidus ‘178 in view of De Luca and Olson Renders Claim 14 Obvious

‘781 Patent, Claim 14	Lapidus ‘178 in view of De Luca, Olson, and Leung
14. The method of claim 3, wherein testing for an amount of a blood protein in the removed portion comprises testing for a concentration of hemoglobin in the removed portion.	Lapidus ‘178 discloses that after “a circumferential surface or cross-sectional sample of stool is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate,

	to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer.” Ex. 1002 at column 6, lines 45-56. Lapidus ‘178 discloses that “an assay performed on at least a circumferential surface or cross-sectional portion of stool may be an assay to detect the presence of elevated levels of carcinoembryonic antigen shed from cells lining the colon. Such an assay may also comprise detecting the presence of occult blood.” <i>Id.</i> at column 3, lines 26-30. Lapidus ‘178 discloses that “[i]f a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art.” <i>Id.</i> at column 9, lines 40-45. De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more
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	analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, Lapidus ‘178 discloses removing a portion of the fecal sample to produce a removed portion. As discussed *supra*, the ‘removed portion’ could take a number of forms, a cross-sectional portion, a circumferential surface portion, and/or a layer of the voided stool sample. Lapidus ‘178 then describes that an assay can be performed on the removed portion to detect the presence of elevated levels of carcinoembryonic antigen (i.e., CEA), which is a known blood protein and is evaluated to detect the presence of occult blood. Furthermore, Lapidus ‘178 discloses that “[i]f a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art.” A person of ordinary skill in the art would understand that such fecal occult blood assays

test for an amount of a blood protein (hemoglobin) that is present in a sample. Ex. 1014 at paragraph 197.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. *Id.* at paragraph 201. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Lapidus '178 in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* at paragraph 201.

Because the other limitations of claim 14 would have been obvious for the reasons explained above for claim 3, claim 14 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '178 in view of De Luca and Olson.

2. Lapidus '650 in view of De Luca and Olson Renders Claim 14 Obvious

'781 Patent, Claim 14	Lapidus '650 in view of De Luca, Olson, and Leung
14. The method of claim 3, wherein testing for an amount of a blood protein in the removed portion comprises testing for a concentration of hemoglobin in the removed portion.	Lapidus '650 discloses “[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics

	indicative of colorectal cancer”. Ex. 1003 at column 6, lines 1-11. Lapidus ‘650 discloses that “an assay performed on at least a cross-sectional portion of stool may be an assay to detect the presence of elevated levels of carcinoembryonic antigen shed from cells lining the colon. Such an assay may also comprise detecting the presence of occult blood.” <i>Id.</i> at column 3, lines 4-9. Lapidus ‘650 discloses that “[i]f a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art.” <i>Id.</i> at column 8, lines 13-18. De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces
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	samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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Lapidus ‘650 discloses removing a portion of the fecal sample to produce a removed portion. As discussed *supra*, the ‘removed portion’ could take a number of forms, a cross-sectional portion, a circumferential surface portion, and/or a layer of the voided stool sample. Lapidus ‘650 then discloses that an assay can be performed on the removed portion to detect the presence of elevated levels of carcinoembryonic antigen (i.e., CEA), which is a known blood protein. Further, Lapidus ‘650 discloses that an assay can be performed on the removed portion to detect the presence of occult blood. “Current non-invasive diagnostic methods involve assaying stool samples for the presence of fecal occult blood or for elevated levels of carcinoembryonic antigen, both of which are suggestive of the presence of colorectal cancer.” *Id.* at column 1, lines 55-58. A person of ordinary skill in the art would understand that such fecal occult blood assays test for an amount of a blood protein (hemoglobin) that is present in a sample. Ex. 1014 at paragraph 197.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. *Id.* at paragraph 201. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Lapidus ‘650 in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the ‘781 Patent. *Id.* at paragraph 201.

Because the other limitations of claim 14 would have been obvious for the reasons explained above for claim 3, claim 14 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '650 in view of De Luca and Olson.

3. Ahlquist in view of De Luca and Olson Renders Claim 14 Obvious

'781 Patent, Claim 14	Ahlquist in view of De Luca and Olson
14. The method of claim 3, wherein testing for an amount of a blood protein in the removed portion comprises testing for a concentration of hemoglobin in the removed portion.	Ahlquist discloses for occult blood testing that “[t]he manufacturer that developed the Hemoccult and HemoccultSensa cards, without rehydration, trained technicians on-site at the Mayo Clinic. As recommended by the manufacturer, the technicians added the catalyst solution to cards stored at ambient temperature within 48 to 72 hours of collection. We defined a spreading (enlarging) blue color in 60 seconds in any window of the cards as a positive result and any other result as negative.” Ex. 1004 at page 5, at heading ‘Occult Blood Testing.’ Ahlquist discloses that “[d]etection sensitivities for the 290 screen-relevant neoplasms found among all 3764 evaluable participants were 10% (95% CI, 7% to 13%) with Hemoccult and 18% (CI, 13% to 22%) with HemoccultSensa (P < 0.001). Based on all 3474 participants without screenrelevant neoplasia, the Hemoccult specificity of 98% (CI, 98% to 99%) was slightly higher than that of HemoccultSensa (97% [CI, 96% to 97%]) (P < 0.001). Hemoccult and

	HemoccultSensa positivity rates for the 39 patients with colorectal cancer or high-grade dysplasia were 33% (CI, 19% to 48%) and 44% (CI, 28% to 59%), respectively ($P = 0.100$). For the 251 patients with adenomas 1 cm or larger, the positivity rates were 6% (CI, 3% to 9%) versus 14% (CI, 9% to 18%) ($P = 0.001$).” <i>Id.</i> at pages 6-y, under heading ‘Occult Blood Testing: Hemoccult versus HemoccultSensa.’ De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of
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	the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 3. Further, Ahlquist discloses testing the removed portion of the fecal sample for a concentration of hemoglobin in the removed portion. As discussed *supra*, Ahlquist discloses that the patients collected stool and then “smeared stool” onto Hemoccult and HemoccultSensa cards. This smeared stool sample corresponds to the ‘removed portion’ of the fecal sample. Further, Ahlquist discloses that occult blood testing was performed on the Hemoccult and HemoccultSensa cards. It is well known in the art that occult blood testing refers to analyzing for the presence of blood proteins such as hemoglobin. Ex. 1014 at paragraph 199.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for a blood protein by utilizing the fecal immunochemical blood tests taught by De Luca and as suggested by Ahlquist instead of the guaiac-based Hemoccult and HemoccultSensa tests of Ahlquist. Ex. 1014 at paragraph 201. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests.

Because the other limitations of claim 14 would have been obvious for the reasons explained above for claim 3, claim 14 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca and Olson.

4. O’Neill in view of De Luca and Olson Renders Claim 14 Obvious

‘781 Patent, Claim 14	O’Neill in view of De Luca, and Olson
14. The method of claim 3, wherein testing for an amount of a blood protein in the removed portion comprises testing for a concentration of hemoglobin in the removed portion.	O’Neill discloses “a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools.” Ex. 1005 at page 1, lines 5-7. O’Neill discloses that “[e]xfoliated 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.” <i>Id.</i> at page 1, lines 15-24. De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom
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	cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, O’Neill in view of De Luca and Olson disclose the limitations of claim 3. O’Neill discloses testing the removed portion of cellular material from the fecal sample for various components, such as DNA, RNA, and proteins. It is well known in the art that testing such components from fecal samples could include analysis of hemoglobin via occult blood testing, fecal immunochemical blood tests, and the like for evaluating colorectal cancer in patients. Ex. 1014 at paragraphs 198 and 200-201.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. Ex. 1014 at paragraph 201. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that O’Neill in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the ‘781 Patent. *Id.*

Because the other limitations of claim 14 would have been obvious for the reasons explained above for claim 3, claim 14 also would have been obvious to a person of ordinary skill in the art in view of O’Neill in view of De Luca and Olson.

O. Dependent Claim 15

1. Lapidus ‘178 in view of De Luca and Olson Renders Claim 15 Obvious

‘781 Patent, Claim 15	Lapidus ‘178 in view of De Luca and Olson
15. The method of claim 14, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.	Lapidus ‘178 discloses that after “a circumferential surface or cross-sectional sample of stool is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the

	sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer.” Ex. 1002 at column 6, lines 45-56. Lapidus ‘178 discloses that “an assay performed on at least a circumferential surface or cross-sectional portion of stool may be an assay to detect the presence of elevated levels of carcinoembryonic antigen shed from cells lining the colon. Such an assay may also comprise detecting the presence of occult blood.” <i>Id.</i> at column 3, lines 26-30. Lapidus ‘178 discloses that “[i]f a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art.” <i>Id.</i> at column 9, lines 40-45. De Luca discloses “collection of faeces samples, particularly for laboratory
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	immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 14. Lapidus ‘178 discloses testing the removed portion of cellular material from the fecal sample for various components, such as DNA, RNA, and proteins. It is well known in the art that testing such components from fecal samples could include analysis of hemoglobin via occult blood testing, fecal immunochemical blood tests, and the like for evaluating colorectal cancer in patients. Ex. 1014 at paragraph 207.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. *Id.* at paragraphs 208 and 211. To this end, De

Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Lapidus '178 in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.*

Because the other limitations of claim 15 would have been obvious for the reasons explained above for claim 14, claim 15 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '178 in view of De Luca and Olson.

2. Lapidus '650 in view of De Luca and Olson Renders Claim 15 Obvious

'781 Patent, Claim 15	Lapidus '650 in view of De Luca and Olson
15. The method of claim 14, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.	Lapidus '650 discloses “[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer”. <i>Id.</i> at column 6, lines 1-11. Lapidus '650 discloses that “an assay performed on at least a cross-sectional portion of stool may be an assay to detect the presence of elevated levels of carcinoembryonic antigen shed from cells

	lining the colon. Such an assay may also comprise detecting the presence of occult blood.” <i>Id.</i> at column 3, lines 4-9. Lapidus ‘650 discloses that “[i]f a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers useful for detecting the presence of fecal occult blood are known in the art.” <i>Id.</i> at column 8, lines 13-18. De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom
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	cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 14. Lapidus ‘650 discloses testing the removed portion of cellular material from the fecal sample for various components, such as DNA, RNA, and proteins. It is well known in the art that testing such components from fecal samples could include analysis of hemoglobin via occult blood testing, fecal immunochemical blood tests, and the like for evaluating colorectal cancer in patients. Ex. 1014 at paragraph 207.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. *Id.* at paragraphs 208 and 211. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that Lapidus ‘650 in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the ‘781 Patent. *Id.*

Because the other limitations of claim 15 would have been obvious for the reasons explained above for claim 14, claim 15 also would have been obvious to a person of ordinary skill in the art in view of Lapidus ‘650 in view of De Luca and Olson.

3. Ahlquist in view of De Luca and Olson Renders Claim 15 Obvious

‘781 Patent, Claim 15	Ahlquist in view of De Luca and Olson
15. The method of claim 14, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.	Ahlquist discloses for occult blood testing that “[t]he manufacturer that developed the Hemocult and HemocultSensa cards, without rehydration, trained technicians on-site at the Mayo Clinic. As recommended by the manufacturer, the technicians added the

	catalyst solution to cards stored at ambient temperature within 48 to 72 hours of collection. We defined a spreading (enlarging) blue color in 60 seconds in any window of the cards as a positive result and any other result as negative.” Ex. 1004 at page 5, at heading ‘Occult Blood Testing.’ Ahlquist discloses that “[d]etection sensitivities for the 290 screen-relevant neoplasms found among all 3764 evaluable participants were 10% (95% CI, 7% to 13%) with Hemoccult and 18% (CI, 13% to 22%) with HemoccultSensa ($P < 0.001$). Based on all 3474 participants without screenrelevant neoplasia, the Hemoccult specificity of 98% (CI, 98% to 99%) was slightly higher than that of HemoccultSensa (97% [CI, 96% to 97%]) ($P < 0.001$). Hemoccult and HemoccultSensa positivity rates for the 39 patients with colorectal cancer or high-grade dysplasia were 33% (CI, 19% to 48%) and 44% (CI, 28% to 59%), respectively ($P = 0.100$). For the 251 patients with adenomas 1 cm or larger, the positivity rates were 6% (CI, 3% to 9%) versus 14% (CI, 9% to 18%) ($P = 0.001$).” <i>Id.</i> at pages 6-y, under heading ‘Occult Blood Testing: Hemoccult versus HemoccultSensa.’ De Luca discloses “collection of faeces samples, particularly for laboratory
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	immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. Further, Ahlquist discloses testing the removed portion of the fecal sample for a concentration of hemoglobin in the removed portion. As discussed *supra*, Ahlquist discloses that the patients collected stool and then “smeared stool” onto Hemoccult and HemoccultSensa cards. This smeared stool sample corresponds to the ‘removed portion’ of the fecal sample. Further, Ahlquist discloses that occult blood testing was performed on the Hemoccult and HemoccultSensa cards. It is well known in the art that occult blood testing refers to analyzing for the presence of blood proteins such as hemoglobin. Ex. 1014 at paragraph 209.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for a blood protein by utilizing the fecal immunochemical blood tests taught by De Luca and as suggested by Ahlquist instead of the guaiac-based Hemoccult and HemoccultSensa tests of Ahlquist. *Id.* at paragraphs 208-209 and 211.

Because the other limitations of claim 15 would have been obvious for the reasons explained above for claim 14, claim 15 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca and Olson.

4. O'Neill in view of De Luca and Olson Renders Claim 15 Obvious

'781 Patent, Claim 15	O'Neill in view of De Luca and Olson
15. The method of claim 14, wherein testing for the concentration of hemoglobin comprises immunochemical detection of hemoglobin.	O'Neill discloses "a method and apparatus for removing cellular material from faecal stools and a container for collecting faecal stools." Ex. 1005 at page 1, lines 5-7. O'Neill discloses that "[e]xfoliated 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." <i>Id.</i> at page 1, lines 15-24.

	De Luca discloses “collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin...”. Ex. 1006 at [0001]. De Luca discloses “[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. <i>Id.</i> at [0019]. Further, De Luca discloses that “the extraction tube [] is positioned in a special housing [] of an automatic machine for immunological tests and then the bottom cap [] is removed so as to allow removal of the buffer solution [] containing the faeces samples by a probe of the automatic machine.” <i>Id.</i> at [0048].
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As discussed *supra*, O’Neill in view of De Luca and Olson disclose the limitations of claim 14. O’Neill discloses testing the removed portion of cellular material from the fecal sample for various components, such as DNA, RNA, and proteins. It is well known in the art that testing such components from fecal samples could include analysis of hemoglobin via occult blood testing, fecal immunochemical blood tests, and the like for evaluating colorectal cancer in patients. Ex. 1014 at paragraph 210.

Moreover, as discussed *supra*, a person of ordinary skill in the art would have been motivated to test the removed portion of the fecal sample for hemoglobin by utilizing the fecal immunochemical blood tests taught by De Luca. *Id.* at paragraphs 208 and 211. To this end, De Luca also discloses testing a removed portion of the fecal sample for hemoglobin, albeit with the more effective fecal immunochemical blood tests. Therefore, one skilled in the art would recognize that O'Neill in view of De Luca disclose all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* at paragraph 211.

Because the other limitations of claim 15 would have been obvious for the reasons explained above for claim 14, claim 15 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca and Olson.

P. Dependent Claim 16

1. Lapidus '178 in view of De Luca, Olson, and Hoepffner Renders Claim 16 Obvious

'781 Patent, Claim 16	Lapidus '178 in view of De Luca, Olson, and Hoepffner
16. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 5 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus '178 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus '178 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus '178/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based

tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is greater than or equal to 10 ng/ml.

Since Lapidus '178 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result. Ex. 1014 at paragraph 219.

Moreover, a person of ordinary skill in the art would have been motivated to further improve the sensitivity of the hemoglobin detection tests by reducing the amount of identified hemoglobin that indicates a positive test. For instance, based on the teachings of Hoepffner, a skilled artisan would want to reduce the amount of identified hemoglobin that indicates a positive test to as low as possible (e.g., from 10 mg/mL to a value of “at least 5 ng/ml”). Such motivation exists because a skilled artisan would desire to improve disease diagnosis in patients with blood/hemoglobin in their stools. Ex. 1014 at paragraph 219.

Because the other limitations of claim 16 would have been obvious for the reasons explained above for claim 14, claim 16 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '178 in view of De Luca, Olson, and Hoepffner.

2. Lapidus '650 in view of De Luca, Olson, and Hoepffner Renders Claim 16 Obvious

'781 Patent, Claim 16	Lapidus '650 in view of De Luca, Olson, and Hoepffner
16. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 5 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus '650 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus '650 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus '650/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is greater than or equal to 10 ng/ml.

Since Lapidus '650 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result. Ex. 1014 at paragraph 219.

Moreover, a person of ordinary skill in the art would have been motivated to further improve the sensitivity of the hemoglobin detection tests by reducing the amount of identified hemoglobin that indicates a positive test. For instance, based on the teachings of Hoepffner, a skilled artisan would want to reduce the amount of identified hemoglobin that indicates a positive test to as low as possible (e.g., from 10 mg/mL to a value of "at least 5 ng/ml"). Such motivation exists because a skilled artisan would desire to improve disease diagnosis in patients with blood/hemoglobin in their stools. Ex. 1014 at paragraph 219.

Because the other limitations of claim 16 would have been obvious for the reasons explained above for claim 14, claim 16 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '650 in view of De Luca, Olson, and Hoepffner.

3. Ahlquist in view of De Luca, Olson, and Hoepffner Renders Claim 16 Obvious

‘781 Patent, Claim 16	Ahlquist in view of De Luca, Olson, and Hoepffner
16. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 5 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. In particular, Ahlquist and De Luca teach the evaluation of fecal samples discloses testing the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Ahlquist/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Ahlquist and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result. Ex. 1014 at paragraph 219.

Moreover, a person of ordinary skill in the art would have been motivated to further improve the sensitivity of the hemoglobin detection tests by reducing the amount of identified hemoglobin that indicates a positive test. For instance, based on the teachings of Hoepffner, a skilled artisan would want to reduce the amount of identified hemoglobin that indicates a

positive test to as low as possible (e.g., from 10 mg/mL to a value of “at least 5 ng/ml”). Such motivation exists because a skilled artisan would desire to improve disease diagnosis in patients with blood/hemoglobin in their stools. Ex. 1014 at paragraph 219.

Because the other limitations of claim 16 would have been obvious for the reasons explained above for claim 14, claim 16 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca, Olson, and Hoepffner.

4. O'Neill in view of De Luca, Olson, and Hoepffner Renders Claim 16 Obvious

'781 Patent, Claim 16	O'Neill in view of De Luca, Olson, and Hoepffner
16. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 5 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 14. In particular, O'Neill and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, O'Neill/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since O'Neill and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result. Ex. 1014 at paragraph 219.

Moreover, a person of ordinary skill in the art would have been motivated to further improve the sensitivity of the hemoglobin detection tests by reducing the amount of identified hemoglobin that indicates a positive test. For instance, based on the teachings of Hoepffner, a skilled artisan would want to reduce the amount of identified hemoglobin that indicates a positive test to as low as possible (e.g., from 10 mg/mL to a value of “at least 5 ng/ml”). Such motivation exists because a skilled artisan would desire to improve disease diagnosis in patients with blood/hemoglobin in their stools. Ex. 1014 at paragraph 219.

Because the other limitations of claim 16 would have been obvious for the reasons explained above for claim 14, claim 16 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca, Olson, and Hoepffner.

Q. Dependent Claim 17

1. Lapidus ‘178 in view of De Luca, Olson, and Hoepffner Renders Claim 17 Obvious

‘781 Patent, Claim 17	Lapidus ‘178 in view of De Luca, Olson, and Hoepffner
17. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 10 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘178 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating

proteins such as hemoglobin. However, Lapidus '178/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus '178 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 17 would have been obvious for the reasons explained above for claim 14, claim 17 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '178 in view of De Luca, Olson, and Hoepffner.

2. Lapidus '650 in view of De Luca, Olson, and Hoepffner Renders Claim 17 Obvious

'781 Patent, Claim 17	Lapidus '650 in view of De Luca, Olson, and Hoepffner
17. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 10 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘650 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus ‘650/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus ‘650 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 17 would have been obvious for the reasons explained above for claim 14, claim 17 also would have been obvious to a person of ordinary skill in the art in view of Lapidus ‘650 in view of De Luca, Olson, and Hoepffner.

3. Ahlquist in view of De Luca, Olson, and Hoepffner Renders Claim 17 Obvious

‘781 Patent, Claim 17	Ahlquist in view of De Luca, Olson, and Hoepffner
17. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 10 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. In particular, Ahlquist and De Luca teach the evaluation of fecal samples discloses testing the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Ahlquist/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Ahlquist and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 17 would have been obvious for the reasons explained above for claim 14, claim 17 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca, Olson, and Hoepffner.

4. O'Neill in view of De Luca, Olson, and Hoepffner Renders Claim 17 Obvious

'781 Patent, Claim 17	O'Neill in view of De Luca, Olson, and Hoepffner
17. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 10 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 14. In particular, O'Neill and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, O'Neill/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since O'Neill and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 17 would have been obvious for the reasons explained above for claim 14, claim 17 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca, Olson, and Hoepffner.

R. Dependent Claim 18

1. Lapidus '178 in view of De Luca, Olson, and Hoepffner Renders Claim 18 Obvious

'781 Patent, Claim 18	Lapidus '178 in view of De Luca, Olson, and Hoepffner
18. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin	Hoepffner discloses that "the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off

detected in the removed portion is at least 20 ng/ml.	<10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘178 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus ‘178/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus ‘178 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

2. Lapidus ‘650 in view of De Luca, Olson, and Hoepffner Renders Claim 18 Obvious

‘781 Patent, Claim 18	Lapidus ‘650 in view of De Luca, Olson, and Hoepffner
18. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148,

detected in the removed portion is at least 20 ng/ml.	second column, first full paragraph (emphasis added).
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As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘650 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus ‘650/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus ‘650 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

3. Ahlquist in view of De Luca, Olson, and Hoepffner Renders Claim 18 Obvious

‘781 Patent, Claim 18	Ahlquist in view of De Luca, Olson, and Hoepffner
18. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 20 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148,

	second column, first full paragraph (emphasis added).
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As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. In particular, Ahlquist and De Luca teach the evaluation of fecal samples discloses testing the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Ahlquist/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Ahlquist and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 18 would have been obvious for the reasons explained above for claim 14, claim 18 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca, Olson, and Hoepffner.

4. O'Neill in view of De Luca, Olson, and Hoepffner Renders Claim 18 Obvious

'781 Patent, Claim 18	O'Neill in view of De Luca, Olson, and Hoepffner
18. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148,

detected in the removed portion is at least 20 ng/ml.	second column, first full paragraph (emphasis added).
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As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 14. In particular, O'Neill and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, O'Neill/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since O'Neill and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 18 would have been obvious for the reasons explained above for claim 14, claim 18 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca, Olson, and Hoepffner.

S. Dependent Claim 19

1. Lapidus '178 in view of De Luca, Olson, and Hoepffner Renders Claim 19 Obvious

'781 Patent, Claim 19	Lapidus '178 in view of De Luca, Olson, and Hoepffner
19. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood	Hoepffner discloses that "the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test,

when the concentration of hemoglobin detected in the removed portion is at least 50 ng/ml.	the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).
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As discussed *supra*, Lapidus ‘178 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘178 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus ‘178/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus ‘178 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 19 would have been obvious for the reasons explained above for claim 14, claim 19 also would have been obvious to a person of ordinary skill in the art in view of Lapidus ‘178 in view of De Luca, Olson, and Hoepffner.

2. Lapidus ‘650 in view of De Luca, Olson, and Hoepffner Renders Claim 19 Obvious

‘781 Patent, Claim 19	Lapidus ‘650 in view of De Luca, Olson, and Hoepffner
19. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 50 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus ‘650 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus ‘650 and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, Lapidus ‘650/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus ‘650 and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 19 would have been obvious for the reasons explained above for claim 14, claim 19 also would have been obvious to a person of ordinary skill in the art in view of Lapidus ‘650 in view of De Luca, Olson, and Hoepffner.

3. Ahlquist in view of De Luca, Olson, and Hoepffner Renders Claim 19 Obvious

‘781 Patent, Claim 19	Ahlquist in view of De Luca, Olson, and Hoepffner
19. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 50 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. In particular, Ahlquist and De Luca teach the evaluation of fecal samples discloses testing the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Ahlquist/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Ahlquist and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 19 would have been obvious for the reasons explained above for claim 14, claim 19 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca, Olson, and Hoepffner.

4. O'Neill in view of De Luca, Olson, and Hoepffner Renders Claim 19 Obvious

'781 Patent, Claim 19	O'Neill in view of De Luca, Olson, and Hoepffner
19. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 50 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 14. In particular, O'Neill and De Luca teach the evaluation of fecal samples includes testing the removed portion of the fecal sample for the presence of blood by evaluating proteins such as hemoglobin. However, O'Neill/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since O'Neill and De Luca teach evaluation of proteins such as hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 19 would have been obvious for the reasons explained above for claim 14, claim 19 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca, Olson, and Hoepffner.

T. Dependent Claim 20

1. Lapidus '178 in view of De Luca, Olson, and Hoepffner Renders Claim 20 Obvious

'781 Patent, Claim 20	Lapidus '178 in view of De Luca, Olson, and Hoepffner
20. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 200 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus '178 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus '178 and De Luca teach the evaluation of fecal samples includes proteins such as hemoglobin the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Lapidus '178/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of proteins such as hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus '178 and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult

Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 20 would have been obvious for the reasons explained above for claim 14, claim 20 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '178 in view of De Luca, Olson, and Hoepffner.

2. Lapidus '650 in view of De Luca, Olson, and Hoepffner Renders Claim 20 Obvious

'781 Patent, Claim 20	Lapidus '650 in view of De Luca, Olson, and Hoepffner
20. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 200 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Lapidus '650 in view of De Luca and Olson disclose the limitations of claim 14. In particular, Lapidus '650 and De Luca teach the evaluation of fecal samples includes proteins such as hemoglobin the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Lapidus '650/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of proteins such as hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Lapidus '650 and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 20 would have been obvious for the reasons explained above for claim 14, claim 20 also would have been obvious to a person of ordinary skill in the art in view of Lapidus '650 in view of De Luca, Olson, and Hoepffner.

3. Ahlquist in view of De Luca, Olson, and Hoepffner Renders Claim 20 Obvious

'781 Patent, Claim 20	Ahlquist in view of De Luca, Olson, and Hoepffner
20. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 200 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 14. In particular, Ahlquist and De Luca teach the evaluation of fecal samples discloses testing the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, Ahlquist/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

Hoepffner removes this deficiency. Hoepffner analyzes concentration of hemoglobin in fecal samples obtained from human patients via various methodologies such as guaiac-based tests, bedside-based immunochemical faecal occult blood tests (IFOBTs), and laboratory-based IFOBTs that use an enzyme linked immunosorbent assay (ELISA) reader. Hoepffner discloses

that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since Ahlquist and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 20 would have been obvious for the reasons explained above for claim 14, claim 20 also would have been obvious to a person of ordinary skill in the art in view of Ahlquist in view of De Luca, Olson, and Hoepffner.

4. O'Neill in view of De Luca, Olson, and Hoepffner Renders Claim 20 Obvious

'781 Patent, Claim 20	O'Neill in view of De Luca, Olson, and Hoepffner
20. The method of claim 14, wherein the removed portion of the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at least 200 ng/ml.	Hoepffner discloses that “the sensitivity of [various] tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively.” Ex. 1009, p. 148, second column, first full paragraph (emphasis added).

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 14. In particular, O'Neill and De Luca teach the evaluation of fecal samples includes proteins such as hemoglobin the removed portion of the fecal sample for the presence of blood by evaluating hemoglobin. However, O'Neill/De Luca do not teach a concentration of hemoglobin that is associated with a positive result.

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Hoepffner discloses that the fecal sample is considered positive for the presence of blood when the concentration of hemoglobin detected in the removed portion is at 10 ng/ml.

Since O'Neill and De Luca teach evaluation of hemoglobin to determine a positive blood protein test, a person of ordinary skill in the art would have been motivated to consult Hoepffner to determine a particular concentration of hemoglobin corresponding to a positive result, wherein the concentration is greater than or equal to 10 ng/ml. Ex. 1014 at paragraph 219.

Because the other limitations of claim 20 would have been obvious for the reasons explained above for claim 14, claim 20 also would have been obvious to a person of ordinary skill in the art in view of O'Neill in view of De Luca, Olson, and Hoepffner.

VIII. CONCLUSION

For at least the reasons set forth above it is respectfully submitted that the prior art cited in this Request present substantial new questions of patentability with respect to claims 1-20 of the '781 Patent. Accordingly, it is earnestly requested that the Office declare a reexamination proceeding of claims 1-20 of the '781 Patent and reject them under at least one of the grounds of rejection set forth above.

In the event there are any unresolved issues that may readily be handled by telephone the Examiner or other Office personnel are kindly invited to contact Requester's undersigned attorney by telephone at (317) 231-6410 so that such issues may be resolved as expeditiously as possible.

Respectfully submitted,

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Date: May 22, 2023

Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of Patients With Colorectal Cancer or Polyps

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Institute of Digestive Disease, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

- BACKGROUND:** Detection of fecal DNA is a promising approach to colorectal cancer screening. However, the sensitivity of current fecal DNA tests for colorectal polyps is low. We evaluated the feasibility of detecting aberrantly methylated DNA or cyclooxygenase-2 (COX-2) mRNA in feces of patients with colorectal cancer or polyps.
- METHODS:** Fecal samples were collected prior to colonoscopy from 20 patients with colorectal cancer, 30 patients with colorectal polyps, and 30 subjects with normal colonic examination. Presence of hypermethylated DNA in 7 tumor-related genes (*APC*, *ATM*, *hMLH1*, *sFRP2*, *HLTF*, *MGMT*, and *GSTP1*) in stool was analyzed by methylation-specific PCR. COX-2 mRNA in fecal samples was detected by RT-PCR.
- RESULTS:** With the use of this panel of methylation markers, the sensitivity of detecting colorectal cancer and adenoma was 75% (95% CI 50.9–91.3%) and 68% (95% CI 46.5–85.1%), respectively. Three normal subjects also had methylated DNA detected in stool, which gives a specificity of 90% (95% CI 73.5–97.9%). The mean number of genes methylated in DNA from the stool of patients with colorectal cancer and adenoma was 1.4 and 0.9, respectively. In contrast, COX-2 mRNA was detected in the stool samples of 10 (50%) cancer patients and one (4%) patient with advanced adenoma only. Two (6.7%) stool samples from normal subjects also had COX-2 mRNA detected.
- CONCLUSION:** Detection of aberrantly methylated DNA in fecal samples is more sensitive than COX-2 mRNA for detection of colorectal cancer and adenoma.

(Am J Gastroenterol 2007;102:1070–1076)

INTRODUCTION

Colorectal cancer is the second most common cancer in the United States (1) as well as in Hong Kong (2). There are approximately 950,000 new cases being diagnosed in the world each year with 480,000 patients dying from this cancer at the same time (3). Because most colorectal cancers follow the adenoma-carcinoma sequence, early detection and removal of adenoma has been shown to reduce the incidence and mortality of colorectal cancer (4).

Among various screening tests for colorectal neoplasms, fecal occult blood testing (FOBT) is generally considered to be the best noninvasive screening test for colorectal cancer. It is the only screening test that is supported by long-term randomized controlled clinical trials to reduce cancer-related mortality (5). However, the sensitivity of FOBT is quite low (15–35%), which implies that a substantial proportion of colorectal neoplasms may be missed (6). In addition, the peroxi-

dase activity present in dietary components may lead to false-positive results, which mandates the need for special dietary restrictions prior to stool collection. With a better understanding of molecular changes associated with colorectal cancer development, there is intense interest in searching for tumor-derived DNA alterations in stool as a noninvasive molecular screening test for colorectal neoplasms. Studies that focus on the detection of mutated DNA in stool have shown encouraging results for the detection of colorectal cancer (7–11). However, the sensitivity in identifying colorectal adenoma remains suboptimal and the positive rate for patients with advanced adenoma is only 15% (11).

Epigenetic changes mediated by promoter hypermethylation of tumor-related genes are increasingly recognized to play an instrumental role in cancer development (12). With the use of multiple methylation markers, virtually all colorectal cancers harbor these epigenetic changes (13). Unlike some genetic mutations that are found in advanced neoplasms only,

we have shown that epigenetic changes are also frequently detected in colorectal adenoma and even nonadvanced adenoma (14). Recently, we and others have demonstrated the feasibility of detecting epigenetic changes in DNA from the stool of patients with colorectal cancer (15–18) and polyps (19).

Another novel approach for colorectal cancer screening is the detection of aberrantly expressed RNA in stool. A group of Japanese investigators has demonstrated the potential of detecting cyclooxygenase-2 (COX-2) mRNA, which is frequently overexpressed in colorectal cancer, in the fecal samples of patients with colorectal cancer (20). However, whether COX-2 mRNA is also detected in the stool samples of patients with colorectal polyps remains unknown. In this pilot study, we sought to explore the feasibility of screening for colorectal cancers and polyps in fecal samples using two different molecular approaches: detecting methylated DNA and detecting COX-2 mRNA.

METHODS

Patients and Fecal Samples

Fecal samples were collected from subjects who attended a colonoscopy screening program in Prince of Wales Hospital, Hong Kong (21). They were all asymptomatic subjects aged 50–70 yr. Subjects who had inflammatory bowel diseases, a family history of familial adenomatous polyposis or hereditary nonpolyposis colon cancer, or previous colonic surgery were excluded. All stool samples were collected one day prior to the initiation of bowel preparation for colonoscopy. The preendoscopy stool specimens were stored in patients' household freezers and patients were asked to return the stool samples on the day of endoscopy for long-term storage at -80°C in our laboratory. During colonoscopy, all polypoid lesions were removed or biopsied. The size of the lesion was determined by the opening of a biopsy forcep, and the location of the lesions was determined on withdrawal of the colonoscope. Histology of colonic polyps was according to the World Health Organization Classification (22). Advanced colonic adenoma was defined as size ≥ 1 cm, the presence of villous histology, or high-grade dysplasia (23). Carcinoma was not counted as advanced colonic adenoma in this study. In cases of patients with more than one lesion in the colon, the most advanced lesion was used in subsequent classification.

As a positive control, stool samples were collected from 20 colorectal cancer patients prior to surgical resection and more than 2 wk from the initial colonoscopy. All participants gave informed consent for obtaining stool specimens for the study. The study protocol was approved by the institutional review board of the Chinese University of Hong Kong.

DNA Isolation and Methylation-Specific PCR

All fecal samples were randomly coded before storage and processing. Staff who worked on these samples were unaware of the clinical diagnosis of the patients. DNA was isolated from each stool sample (250 mg) by the QIAamp DNA Stool Mini kit (Qiagen, Valencia, CA). The quality of human DNA recovered from the stool samples was further verified by PCR

amplification of the human β -globulin gene. With our storage protocol, human β -globulin DNA was successfully amplified in all stool samples.

The presence of methylated DNA in stool was detected by methylation-specific PCR (MSP). Briefly, 2 μg of DNA was chemically modified to convert all unmethylated cytosine to uracil by the EZ DNA methylation kit (Zymo Research, Orange, CA). A total of seven tumor-related genes (*APC*, *ATM*, *HLTF*, *MGMT*, *hMLH-1*, *SFRP2*, and *GSTP1*) were examined as described previously (14–16). This selection was based on previous studies that found that the former six genes were frequently methylated in colorectal cancer (14, 15). The *GSTP1* gene was found to be unmethylated in colorectal cancer and was included as a negative control. CpGenomeTM Universal methylated DNA (Chemicon International Inc., Temecula, CA) was used as a positive control, whereas template-free distilled water was included as a negative control for each amplification. All PCR reactions were duplicated to ensure consistency and reproducibility of the results.

RNA Isolation and RT-PCR

COX-2 expression in stool samples was detected by RT-PCR. Messenger RNA was extracted from stool samples by the QIAamp Viral Mini Spin Kit (Qiagen). All RNA samples were purified with DNase and stored at -80°C after extraction. Complementary DNA (cDNA) was synthesized using random primers and MMLV Reverse Transcriptase (Promega, Madison, WI). RNA integrity was checked by human β -actin mRNA expression. The primers used for β -actin detection were: 5'-CTT CAA CAC CCC AGC CAT GTA CG-3' (forward) and 5'-CAT GAG GTA GTC AGT CAG GTC CCG G-3' (reverse). β -actin mRNA was detected in 77 (96.3%) of the 80 stool samples in this study. The primer sequences for COX-2 were: 5'-TTC AAA TGA GAT TGT GGA AAA ATT GCT-3' (forward) and 5'-AGA TCA TCT CTG CCT GAG TAT CTT-3' (reverse). The cycling conditions were as follows: 95°C for 2 min, followed by 45 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The 195-bp β -actin and 305-bp COX-2 PCR products were identified by agarose gel electrophoresis. All samples were reverse-transcribed and amplified in duplicate to ensure reproducibility. Colon cancer tissue with high COX-2 expression was used as a positive control, whereas template-negative water was included in all amplifications as a negative control.

Statistical Analysis

The sensitivity and specificity (with 95% confidence interval) of the stool assay were computed. Categorical data were analyzed by Fisher's exact test and numerical values were compared by the student's *t*-test. A *P* value of less than 0.05 was considered to be statistical significant.

RESULTS

Patients

Fecal samples were collected from 20 patients with colorectal cancer (mean age 69 yr), 30 patients with colonic polyps

(mean age 70.5 yr), and 30 age-matched controls with a normal colonic examination. There was no significant difference in age among the three groups. Among the 30 patients with colonic polyps, 25 (83.3%) had adenoma and 5 (16.7%) had hyperplastic polyps. Eight (26.7%) patients had advanced colonic adenoma and 11 (36.7%) patients had more than one polyp in the colon. Sixteen (53.3%) patients had polyp distal to the splenic flexure.

Methylation in Fecal DNA

The β -globulin gene was successfully amplified in all the 80 stool samples. With the exception of *GSTP1*, methylation was frequently detected in the other six genes in patients with colorectal cancer or adenoma (Table 1). The frequency of methylation in DNA from the stool of patients (Table 1) with colorectal cancer, advanced adenoma, or nonadvanced adenoma and normal controls is shown in Figure 1. The most frequently methylated gene in the stool of cancer patients and adenoma patients was *SFRP2* (30%) and *HLTF* (20%), respectively. The overall frequency of methylation tended to be higher in the stool samples of cancer patients than of patients with adenoma: *SFRP2* (30% vs 12%), *ATM* (25% vs 16%), *APC* (20% vs 16%), *hMLH1* (20% vs 12%), and *MGMT* (20% vs 12%). The frequency of *HLTF* methylation was comparable in the stool samples of patients with colorectal cancer and adenoma (20% vs 20%). Among patients with colorectal adenoma, methylation was more frequently detected in the stool of patients with advanced adenoma than with nonadvanced adenoma in *HLTF*, *hMLH1*, and *MGMT* (Fig. 1). Notably, methylation was also detected in patients with hyperplastic polyps only. Methylation of *ATM*, *HLTF*, *hMLH1*, and *SFRP2*

were each detected in the stool sample of one patient with a hyperplastic polyp.

With the use of six methylation markers (excluding *GSTP1*), 15 (75%) cancer patients, 17 (68%) patients with colonic adenoma, 2 (40%) patients with hyperplastic polyps, and 3 (10%) normal controls had methylation detected in DNA from their stool (Fig. 2). Five (62.5%) patients with advanced adenoma and 12 (70.6%) patients with nonadvanced adenoma had methylated DNA detected in their stool. The corresponding sensitivities for patients with colorectal cancer and adenoma with this panel of methylation markers were 75% (95% CI 50.9–91.3%) and 68% (95% CI 46.5–85.1%), respectively. The overall specificity of this panel of methylation markers was 90% (95% CI 73.5–97.9%).

The mean number of methylated genes in DNA from the stool of patients with colorectal cancer or adenoma and controls was 1.4, 0.9, and 0.1, respectively. Three (15%) cancer patients had methylation detected in ≥ 3 genes and 7 (35%) patients had methylation in 2 genes. Among the 25 patients with colonic adenoma, only 1 (4%) had methylation in ≥ 3 genes and 3 (12%) had methylation detected in 2 genes. Two (6.7%) normal controls had methylation in the *SFRP2* gene, whereas one (3.3%) had concurrent methylation in *HLTF* and *SFRP2*.

We further analyzed the characteristics of colorectal adenoma with detection of aberrant methylation in stool samples. The mean numbers of methylated genes in stool samples of patients with advanced adenoma tended to be higher (1.4) than those with nonadvanced adenoma (0.8). There was, however, no association between methylation in DNA from

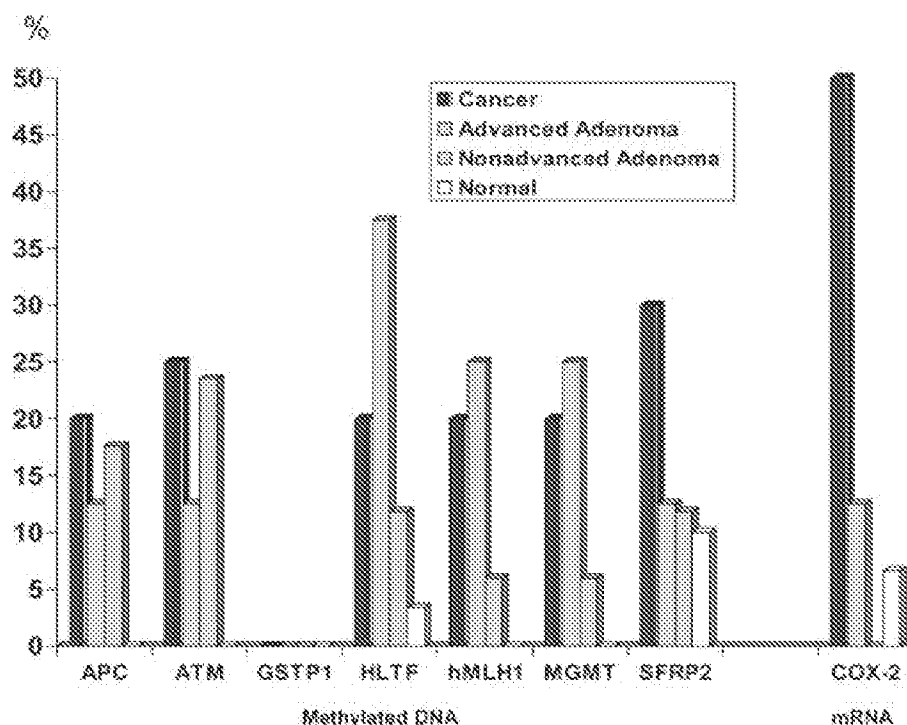


Figure 1. Frequency of detecting methylation changes and COX-2 mRNA in fecal samples.

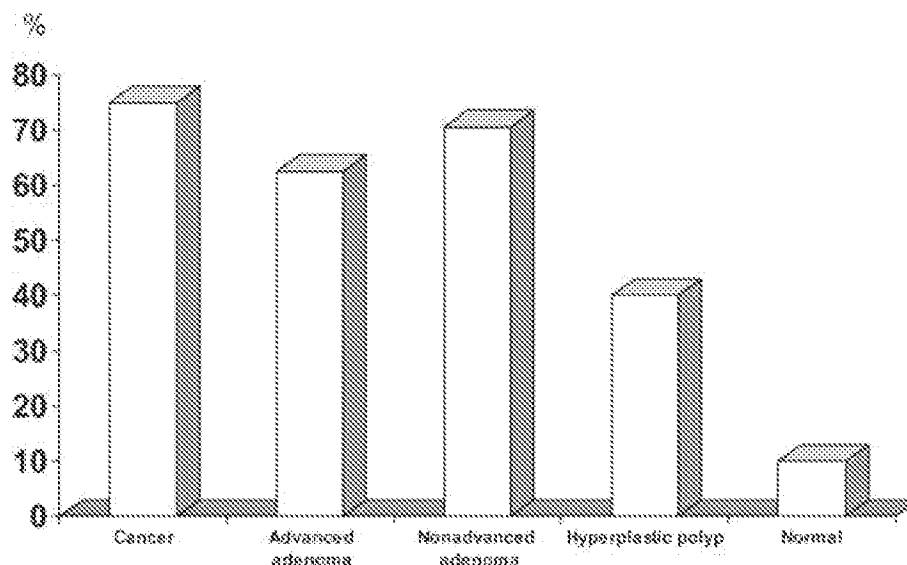


Figure 2. Overall frequency of detecting methylation changes in fecal DNA of patients with different colonic pathology.

stool samples and adenoma location or patient characteristics, including age.

COX-2 mRNA in Feces

We also detected the presence of COX-2 mRNA in these stool samples. β -actin mRNA was successfully amplified in the stool samples of 19 (95%) patients with colorectal cancer, 29 (96.7%) patients with colonic polyps, and 29 (96.7%) control subjects. COX-2 mRNA was detected in 10 (50%) cancer patients, 1 (4%) patient with colonic adenoma, and 2 (6.7%) normal controls. Hence, detection of COX-2 mRNA in stool has a sensitivity of 50% (95% CI 27.2–72.8%) for colorectal cancer and 4% (95% CI 0.1–20.4%) for colorectal adenoma. The specificity for colorectal neoplasm by COX-2 mRNA detection was 93% (95% CI 77.2–99.2%).

To verify the expression levels of COX-2 in tissue samples, we further tested the expression of COX-2 in the primary cancer tissues of the 20 cancer patients and the normal colonic biopsies of the 30 controls. COX-2 mRNA was detected in 14 (70%) cancer samples and 4 (13.3%) normal colonic tissues. Stool samples with COX-2 mRNA expression were all collected from patients with COX-2 mRNA in the corresponding primary tissues.

DISCUSSION

Detection of epigenetic alterations in various body fluids, including serum (24, 25), urine (26), and feces (15–19), is a novel approach for noninvasive cancer detection. We and others have recently demonstrated the feasibility of detecting epigenetic changes or aberrant mRNA expression in stool samples of patients with colorectal cancer (15–19). Herein, we expanded this application to the detection of colorectal polyps through analysis of stool DNA or RNA. Our results showed that methylated DNA was readily detected in the stool samples of patients with colonic adenoma including both ad-

vanced and nonadvanced adenoma. With the use of this panel of six methylation markers, it was found that 75% of cancer and 68% of colonic adenoma patients had methylated DNA detected in their stool samples. The use of methylation markers appears to have a higher sensitivity than the commercially available fecal DNA panel in detecting colorectal adenoma (15.1%) and minor polyps (7.6%) (11). On the other hand, detecting COX-2 mRNA in stool may not be sensitive enough for identification of patients with colonic adenoma (4%).

Unlike previous studies that focus on identification of patients with advanced colonic adenoma (18), we found that methylation could be detected in the DNA from stool samples of patients with hyperplastic polyps (Fig. 2). In contrast, a previous study that used a single methylation marker (HIC1) failed to detect any methylation changes in hyperplastic polyps (18). Recently, Petko *et al.* also demonstrated the feasibility of detecting methylated DNA in the fecal DNA of patients with colorectal polyps (19). However, DNA was isolated from colon lavage effluent during colonoscopy instead of from patients' stool samples. In contrast to our study, a considerable proportion of the fecal DNA from normal subjects also had methylation detected. It remains elusive whether the low specificity is related to the method of sample collection or the choice of methylation markers. Further study is necessary to clarify this discrepancy.

Among the six methylation markers, the sFRP2 methylation marker was previously shown to be highly sensitive for colorectal cancer (15, 18). Although the positive rate of this marker in stool samples of cancer patients was highest among other methylation markers, the inclusion of the sFRP2 methylation marker may impose specificity concerns. In this study, three (10%) subjects with normal colonoscopy had sFRP2 methylation detected in their stool. In keeping with our finding, 23% of normal subjects were found to have sFRP2 methylation in their stool in a previous study (15). The reason for this low specificity may be related to the fact that

Table 1. DNA Methylation and COX-2 mRNA in Fecal Samples of Patients With Colorectal Cancer and Polyps

No.	Sex	Age	Location	Histology	Size(mm)	APC	ATM	HLTF	hMLH1	MGMT	sFRP2	COX2
1	F	71	Descending	CA		—	—	—	—	—	—	—
2	F	45	Splenic	CA		—	—	—	—	+	+	—
3	M	75	Rectum	CA		—	+	+	—	+	—	+
4	F	58	Sigmoid	CA		+	—	—	—	—	—	+
5	M	81	Splenic	CA		—	+	—	—	—	—	—
6	F	85	Descending	CA		+	—	+	—	—	—	+
7	F	84	Ascending	CA		—	+	+	—	—	—	+
8	M	73	Ascending	CA		—	—	—	—	—	—	NA
9	M	87	Rectum	CA		—	—	—	—	—	+	+
10	F	90	Hepatic	CA		—	—	—	+	+	—	—
11	M	66	Sigmoid	CA		—	—	+	+	—	+	—
12	F	54	Cecum	CA		—	+	—	—	—	—	+
13	F	78	Transverse	CA		—	+	+	+	—	—	+
14	F	53	Ascending	CA		+	—	—	—	—	—	—
15	F	55	Ascending	CA		+	—	—	—	—	+	+
16	F	81	Ascending	CA		—	—	—	+	—	+	—
17	M	71	Ascending	CA		—	—	—	—	—	—	—
18	F	69	Rectum	CA		—	—	—	—	+	+	+
19	F	51	Cecum	CA		—	—	—	—	—	—	—
20	M	48	Sigmoid	CA		—	—	—	—	—	—	+
22	F	86	Descending	TV	10	—	—	—	—	—	—	+
24	M	46	Sigmoid	TV	8	+	—	+	+	—	+	NA
25	M	61	Cecum	TV	10	—	—	+	—	—	—	—
30	M	67	Sigmoid	TV	10	—	—	—	—	—	—	—
34	M	80	Ascending	TV	14	—	+	—	—	+	—	—
42	F	81	Ascending	Ad	20	—	—	—	+	+	—	—
44	M	64	Rectum	Ad	20	—	—	—	—	—	—	—
48	F	79	Rectum	TV	15	—	—	+	—	—	—	—
23	F	69	Sigmoid	Ad	5	—	+	—	—	—	—	—
26	M	76	Ascending	Ad	2	—	—	—	—	—	+	—
27	M	76	Sigmoid	Ad	2	—	+	—	—	—	—	—
28	F	53	Transverse	Ad	3	—	—	—	—	—	—	—
31	F	64	Ascending	Ad	2	—	—	—	—	—	—	—
32	F	91	Ascending	Ad	5	—	—	—	—	—	—	—
33	F	68	Sigmoid	Ad	4	—	—	—	—	—	—	—
36	F	87	Ascending	Ad	3	—	—	—	—	—	—	—
37	M	68	Descending	Ad	3	+	—	—	—	—	—	—
38	F	64	Sigmoid	Ad	5	—	+	—	—	—	—	—
39	F	55	Cecum	Ad	2	+	—	—	—	—	—	—
40	M	65	Ascending	Ad	5	—	+	—	—	—	—	—
43	F	66	Transverse	Ad	4	—	—	+	—	—	—	—
45	F	58	Ascending	Ad	5	+	—	—	—	+	—	—
46	F	66	Sigmoid	Ad	2	—	—	+	—	—	—	—
47	F	77	Transverse	Ad	4	—	—	—	+	—	—	—
49	M	81	Rectum	Ad	3	—	—	—	—	—	+	—
21	M	60	Descending	HP	3	—	+	—	—	—	—	—
29	F	88	Transverse	HP	2	—	—	+	+	—	+	—
35	M	73	Rectum	HP	2	—	—	—	—	—	—	—
41	M	82	Sigmoid	HP	2	—	—	—	—	—	—	—
50	M	66	Rectum	HP	3	—	—	—	—	—	—	—

CA = carcinoma; Ad = adenoma; HP = hyperplastic polyp; TV = villous adenoma; NA = mRNA not detected.

sFRP2 methylation could also be detected in aberrant crypt foci, which is not easily detected by ordinary colonoscopy (27). Long-term follow-up of these subjects with methylated sFRP2 in stool samples is necessary to elucidate the true specificity of this methylation marker.

Although methylation is frequently detected in colorectal cancer, it has been demonstrated that methylation changes could also be detected in normal colonic tissues of cancer patients, suggesting the presence of a field effect (28). Hence,

the exact origin of methylated DNA in stool remained uncertain in the absence of follow-up samples collected after surgery or polypectomy. However, because of the rapid turnover of cancer cells, there is a strong reason to believe that methylated DNA in stool is largely derived from neoplastic cells rather than normal colonic cells. In this regard, Klaassen *et al.* have demonstrated the increased concentrations of human DNA in stool samples from colorectal cancer patients (29).

Although COX-2 is frequently expressed in the stool samples of patients with colorectal cancer, the yield of COX-2 mRNA in stool was very low in patients with colonic polyps or adenoma. Apart from a biological reason, the relatively low positive rate of COX-2 mRNA may also be resulting from a technical reason. Whilst RNA is generally less stable than DNA, the method of storage and processing of stool samples may influence the diagnostic yield of PCR. All stool samples were collected by patients at home and then stored at -80°C in our laboratory. The time lag between long-term storage and the subsequent freeze-thaw process may compromise the integrity of RNA and hence the detection rate. It is therefore necessary to develop a better RNA storage and extraction protocol from stool samples to increase the diagnostic yield of this RNA-based detection method.

The detection of methylated DNA or overexpressed mRNA is through the detection of a positive signal. In contrast to the detection of loss of a positive signal, this approach is particularly advantageous in the detection of minute amounts of abnormal DNA or RNA shed in the stool by colonic neoplasms. With the increasing knowledge of molecular pathways leading to colorectal cancer, it is anticipated that more novel target genes will be identified in the future for this purpose. The possibility of multiplex PCR may further enable high-throughput detection of multiple methylation markers. In this study, qualitative MSP was used to detect methylated DNA in stool. Design of primers and preferential amplification of some sequences may affect the sensitivity of MSP. Recently, quantitative PCR has been applied to the detection of methylated DNA (24, 30). The use of quantitative MSP not only improves sensitivity but also provides useful information on the concentration or proportion of methylated DNA in the samples. Intuitively, it may be possible to determine the nature of the colonic lesions (cancer vs adenoma) based on the concentration of methylated DNA. Further study is necessary to establish this possible application.

In conclusion, our results have demonstrated the feasibility of using multiple methylation markers as a noninvasive test for early nonadvanced colorectal polyps. Further study is needed to refine the best panel of methylation markers. On the other hand, the current fecal mRNA assay is not sensitive for identification of patients with colonic adenoma.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Although the fecal occult blood test is a very reliable noninvasive screening test for colorectal cancer, the sensitivity and specificity are generally low.
- Detection of altered DNA in stool appears to be a promising new approach for colorectal cancer screening but the sensitivity for colorectal adenoma detection is unacceptable.

What Is New Here

- We have previously demonstrated the feasibility of detecting methylated DNA in stool samples of patients with colorectal cancer. By using multiple methylation markers, we found that up to 68% of patients with colorectal adenoma could be detected in the present study.
- Although the detection of COX-2 mRNA is the most sensitive single stool marker for colorectal cancer, the sensitivity of detecting COX-2 mRNA in stool samples of patients with colorectal adenoma is very low.

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CONFLICT OF INTEREST

Guarantor of the article: Wai K. Leung, MD

Specific author contributions: Wai K. Leung (Planning and drafting of manuscript); Ka-Fai To (planning and conducting of experiments); Ellen Man & Michael Chan (conducting of experiments); Aric Hui, Simon Ng and James Lau (Sample collection and patients recruitment); Joseph Sung (drafting of manuscript).

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				Art Unit	1634	
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U.S. PATENTS					
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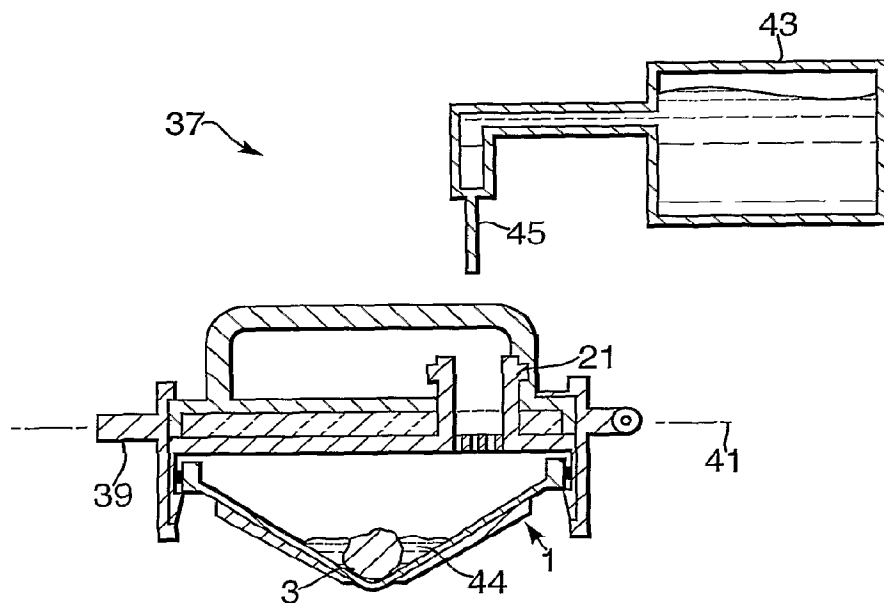
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(54) Title: METHOD AND APPARATUS FOR REMOVING CELLULAR MATERIAL FROM FAECAL STOOLS AND CON-
TAINER FOR COLLECTING FAECAL STOOLS



(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 greazing 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

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

Background

10 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.

15 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
20 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*
25 *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
30 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
35 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.

5 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
10 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
15 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
20 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
25 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
30 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
35 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
5 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
10 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
15 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
20 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
25 (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
30 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
35 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
5 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
10 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
15 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.

20 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
25 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
30 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,
35 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
5 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
10 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
15 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.

20 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
25 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:

30 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).

10 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:

15 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;

20 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;

25 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

30 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
35 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
5 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
10 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
15 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
20 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
25 (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
30 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.

5 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,
10 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
15 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
20 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
25 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
30 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
35 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-
 5 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 μ m instead of a 125 μ m sieve. Cell
 10 clean-up was performed robotically on a TECANTM 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 TECANTM Spectrafluor Plus using PicoGreen
 15 (Molecular ProbesTM) 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
 25 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	stool	stool	stool	stool	stool
	1	2	3	4	5	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

5 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

10 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

15 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

20 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	1 st	2 nd	1 st	2 nd	2 nd	1 st
wash	55	167	234	300	256	49
SDNAI from 0.5	2 nd	1 st	2 nd	1 st	1 st	2 nd
ratio wash	139	92	220	262	207	65

Claims:

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

5 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;

10 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.

15 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.

20 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.

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

30 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

35 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.

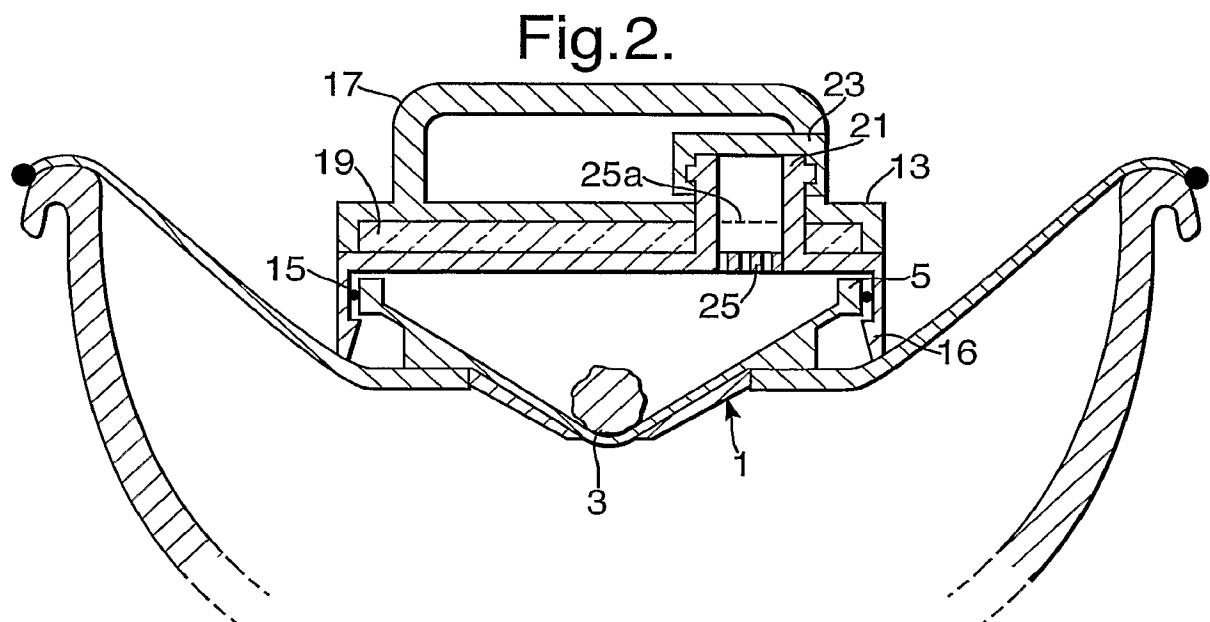
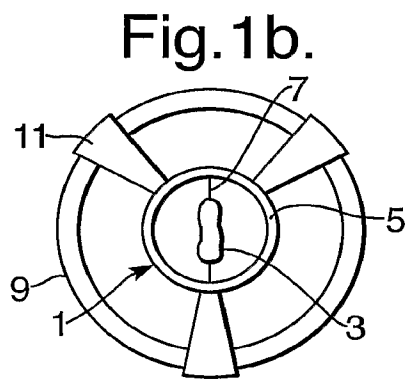
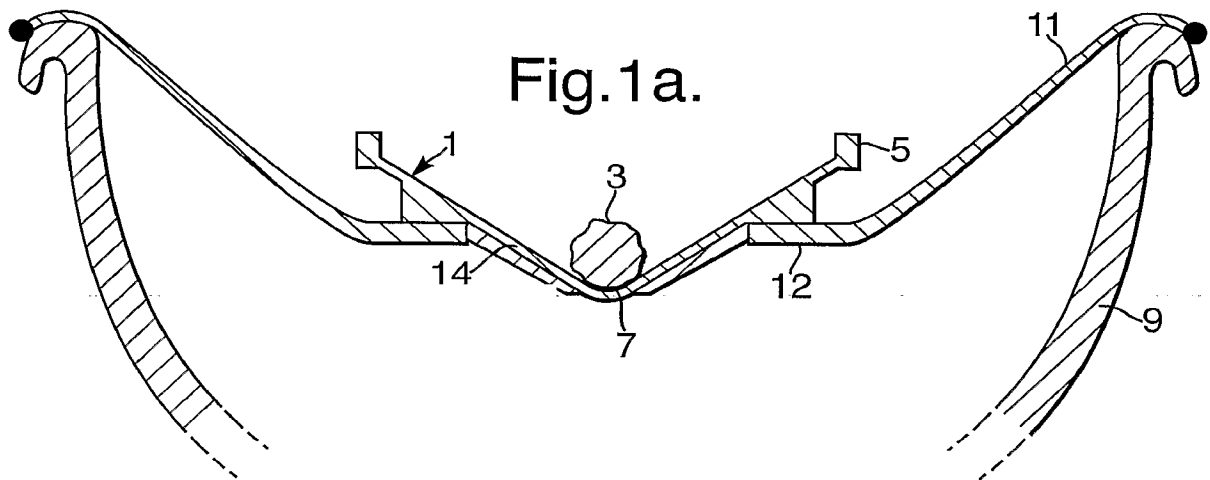
11. A container for collecting faecal stools which has a fixed shape and has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways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

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

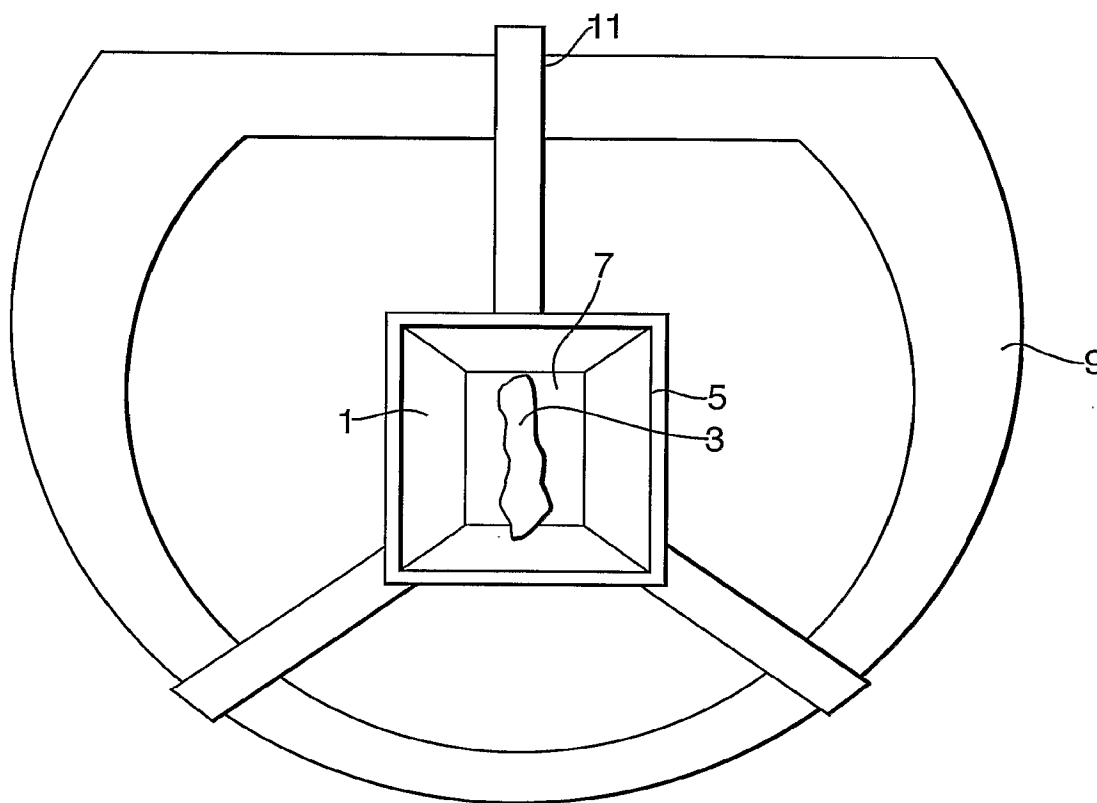
14. A container according to any one of claims 11 to 13,
which is hermetically sealable to reduce the exposure to
atmospheric oxygen of a stool deposited therein.
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Fig. 1c.



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Fig.3a.

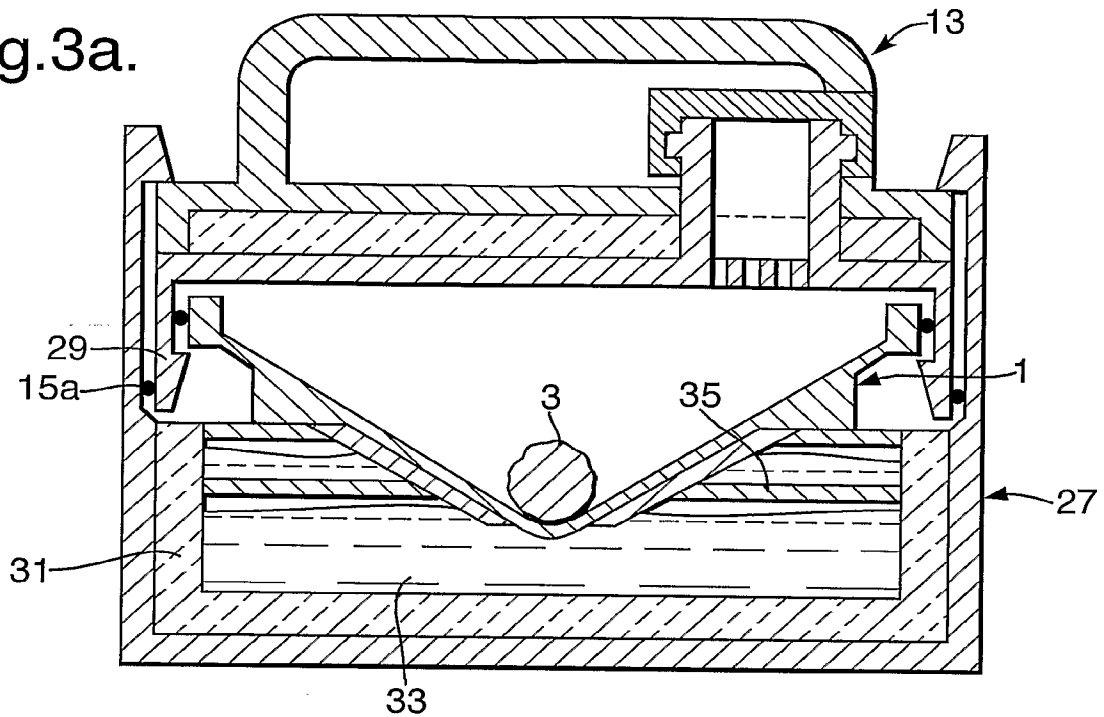
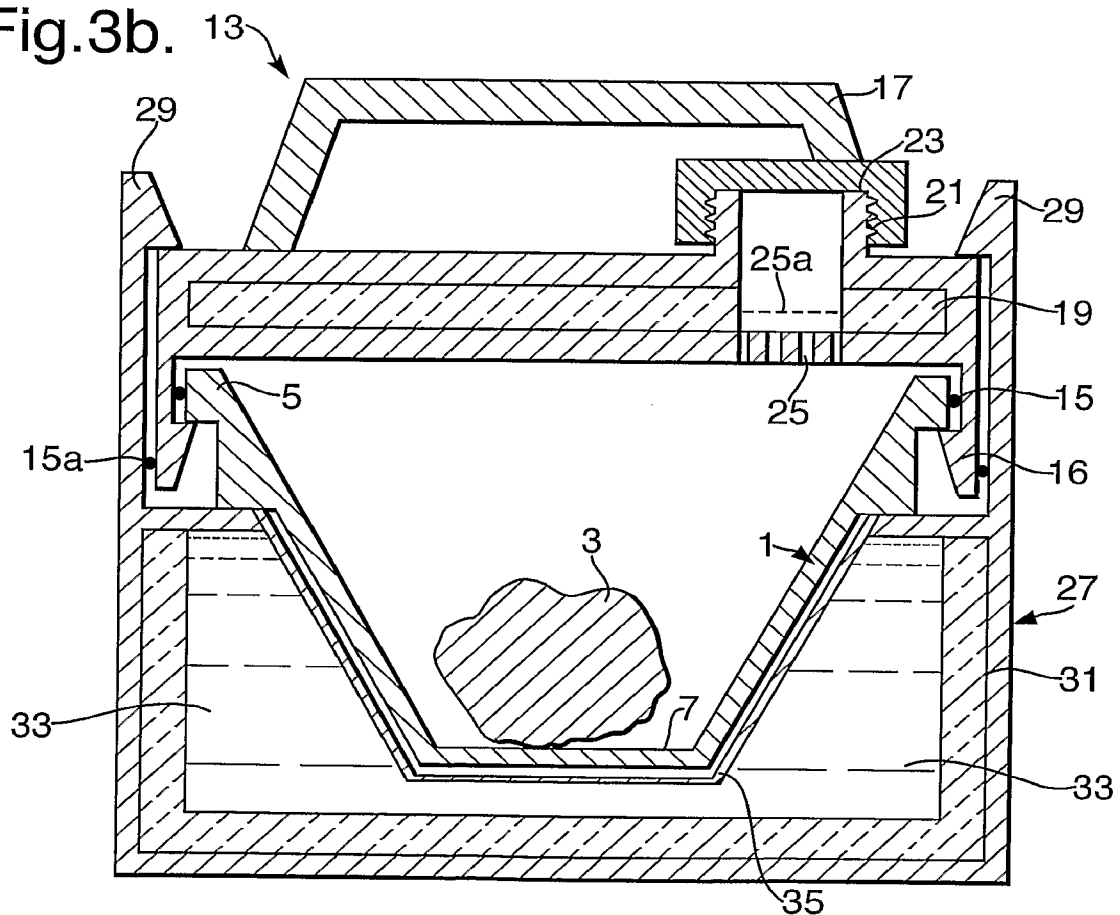
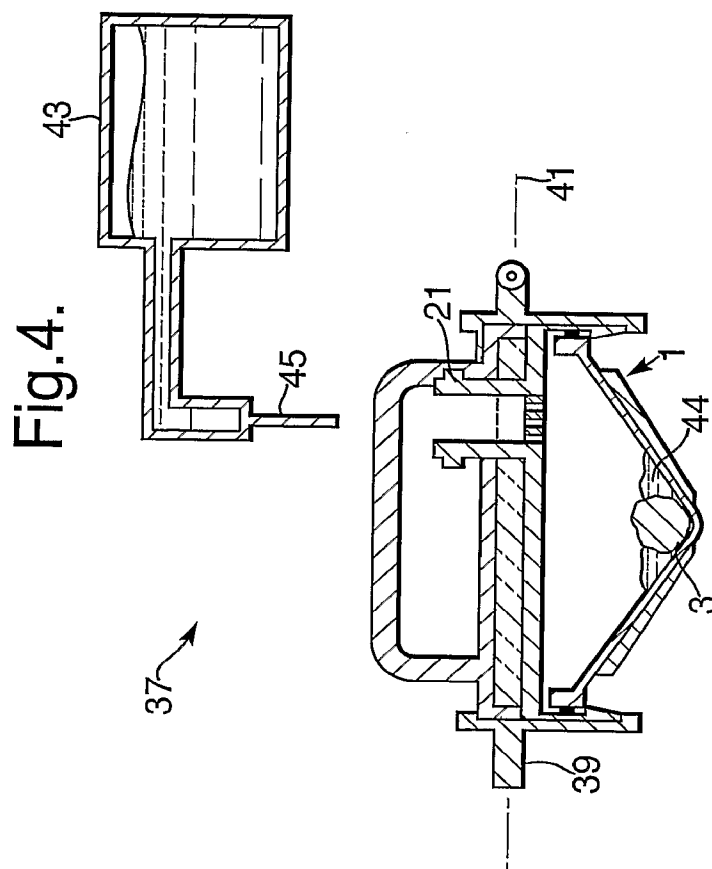
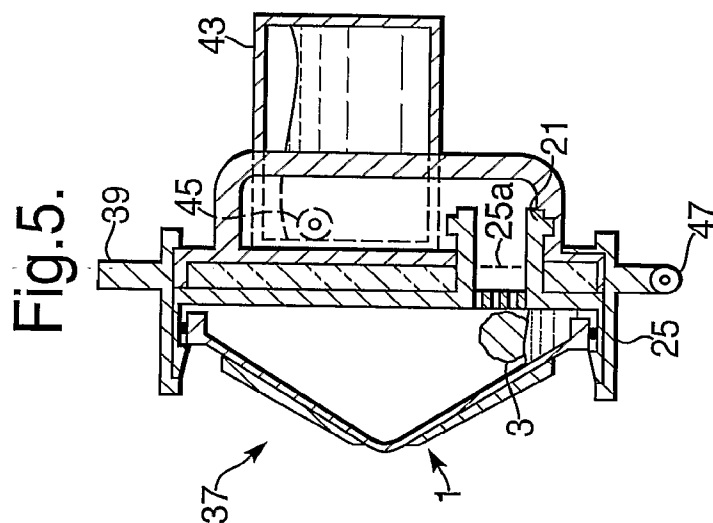


Fig.3b.



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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
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Further documents are listed in the continuation of box C.



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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search

29 October 2004

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #
15/634,607

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06/28/2023 05:19:15 PM ET

ATTORNEY DOCKET #
EXCTD-35239.304

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 1874

FILED BY Stephanie Filandrinos

PATENT CENTER # 62352099

AUTHORIZED BY Mary Ann Brow

CUSTOMER # 173254

FILING DATE 06/27/2017

CORRESPONDENCE ADDRESS -

FIRST NAMED INVENTOR Joost Louwagie

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PAYMENT METHOD
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PAYMENT TRANSACTION ID
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PAYMENT AUTHORIZED BY
Stephanie Filandrinos

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1820	REQUEST FOR CONTINUED EXAMINATION (RCE)- 2ND AND SUBSEQUENT REQUEST (SEE 37 CFR 1.114)	2000.00	1	2000.00
TOTAL AMOUNT:				\$2,000.00

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APPLICATION #
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CUSTOMER # 173254

FIRST NAMED
INVENTOR J oost Louwagie

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Mary Ann Brow

Documents

TOTAL DOCUMENTS: 11

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-06-28_RCE2_EXCTD-003USCON2-35239_35239-304.pdf	3	Request for Continued Examination (RCE)	135 KB
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LEUNG_Amj Gastro_2007.pdf	8	Non Patent Literature	954 KB
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