	containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

Lapidus '650 discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Lapidus '650, the cross-sectional portion corresponds to the 'removed portion' of the fecal sample in independent claim 1.

Given the removal of a cross-sectional sample, logic dictates that Lapidus '650 discloses a 'remaining portion' of the fecal sample that is left over following such removal. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 60-63.

Moreover, Lapidus '650 discloses that the 'removed portion' of the fecal sample is obtained upon collection of the fecal sample. Again referring to Figure 2 above, Lapidus '650 discloses that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." *Id.* at column 7, lines 61-65. This provides evidence that the 'removed portion' of the fecal sample (e.g., a cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

Even if Lapidus '650 is determined to not specifically teach removal to a separate sealable container, De Luca removes this deficiency. De Luca discloses "an extraction and

sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform assays, such as fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a fecal sample with buffer solution and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the 'removed portion' of the fecal sample obtained by Lapidus '650 with the requisite expectation of success. Therefore, one skilled in the art would recognize that Lapidus '650 in view of De Luca discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 66.

'781 Patent, Claim 1, Third Limitation	**Lapidus '650 in view of De Luca and Olson**
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**Lapidus '650** discloses that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline. Homogenization means and materials for homogenization are generally known in the art. Thus, particular homogenization methods may be selected by the skilled artisan and may depend upon the assay to be employed. The buffer may contain detergent, salt, proteinase, inhibitors of DNA and RNA degrading enzymes. The composition of the buffer will depend on the type of assay to be performed. If a fecal occult blood assay is to be performed, the buffer may contain chemical compounds which react with blood to produce a color, the intensity of which can be measured. Buffers

	useful for detecting the presence of fecal occult blood are known in the art. If a test is to be performed for the presence of a particular protein, the buffer should not contain a proteinase capable of degrading such tumor marking antigens." Ex. 1003 at column 8, lines 5-21. **De Luca** discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is

	provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

Lapidus '650 discloses combining the removed portion of the fecal sample (e.g., the cross-sectional portion) with a buffer. Lapidus '650 discloses such buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. Ex. 1014 at paragraphs 72-73. Lapidus '650 further notes that to prevent the denaturation or degradation of blood proteins found in the sample, it would be important to specifically avoid including a proteinase in the buffer that would contain detergents and salts.

Again, Lapidus '650 discloses that the cross-sectional portion of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. This indicates that the removed portion is added to a buffer for further processing.

Even if Lapidus '650 is determined to not specifically teach combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container, De Luca removes this deficiency. De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are used for detection of blood proteins such as hemoglobin for use in fecal immunochemical blood tests. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a buffer solution and fecal sample and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the removed portion from the fecal sample of Lapidus '650. Based on De Luca, a person of ordinary skill in the art would combine the removed portion of the fecal sample in the separate sealable container with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Ex. 1014 at paragraph 75. Further, De

Luca teaches that the extraction tube is sealed using a top cap, rendering the extraction tube to be a "separate sealable container." Therefore, one skilled in the art would recognize that Lapidus '650 in view of De Luca discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Fourth Limitation	**Lapidus '650 in view of De Luca and Olson**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**Lapidus '650** discloses that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1003 at column 2, line 66 – column 3, line 3 (emphasis added). Lapidus '650 discloses that "the receptacle [containing the stool] may comprise means for introducing homogenization buffer or one or more preservatives, such as alcohol, a solution of high salt concentration, antibiotics, and chaotropic salts in order to neutralize bacteria present in the stool sample. The homogenization buffer may be a physiologically compatible buffer such as phosphate buffered saline, and may comprise salt such as 20-100 mM NaCl or KCl. The homogenization buffer may also comprise a detergent, such as 1-10% SDS or triton, and/or a proteinase, such as proteinase K. The buffer may also contain inhibitors of DNA and RNA degrading enzymes. The receptacle, whether adapted to fit a toilet or

	simply adapted for receiving the voided stool sample, should include sealing means sufficient to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." *Id.* at column 7, lines 31-49. **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)."

	Id. at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '650 discloses combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer. Even if Lapidus '650 is determined to not specifically teach combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, Olson removes this deficiency.

Olson discloses that addition of a stabilizing buffer (Exact buffer A) to stool samples provided a significant increase in recovery of DNA compared with samples incubated without buffer. This addition of a stabilizing buffer to DNA led to increased assay sensitivity.

Thus, a person of ordinary skill in the art would have been motivated to combine the remaining portion of the fecal sample with the stabilizing buffer taught by Olson. Olson discloses that DNA assays are greatly improved with addition of stabilizing buffer. Therefore, one skilled in the art would recognize that Lapidus '650 in view of Olson discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 84.

In summary, Lapidus '650 in view of De Luca and Olson disclose the limitations of claim 1 of the '781 Patent. Consequently, claim 1 is obvious over Lapidus '650 in view of De Luca and Olson.

7. Ahlquist in view of De Luca and Olson Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	**Ahlquist in view of De Luca and Olson**
1. A method of processing a freshly-collected fecal sample without freezing, 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 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.

As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, Ahlquist discloses this limitation because Ahlquist teaches collection of stool (a fecal sample) into a plastic bucket. Further, Ahlquist indicates that the stools were frozen ***after*** they were received at the Mayo Clinic. This indicates that the stools were obtained without freezing and therefore a person of ordinary skill in the art would recognize Ahlquist discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. Ex. 1014 at paragraph 48.

'781 Patent, Claim 1, First Limitation	**Ahlquist in view of De Luca and Olson**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**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 collecting a fecal sample from a human subject as it teaches that human patients collected stools (a fecal sample) into a plastic bucket mounted to a toilet seat. This indicates that the human subject defecated directly into the plastic bucket, which is a

collection vessel. Further, Ahlquist teaches that the plastic bucket is a sealable collection vessel because the plastic bucket is later sealed upon shipping.

Further, Ahlquist discloses that the stool can be collected at home according to the broadest reasonable interpretation of the claims consistent with the specification. Ahlquist discloses that the stools are collected by patients and then express-shipped to the Mayo Clinic, meaning that the fecal sample was collected at *a location different than the Mayo Clinic testing facility.* This indicates that the stool can be collected in a toilet that is not located at a testing facility. A person skilled in the art would understand that Ahlquist effectively describes collection of a stool at home, given that "home" is not a testing facility such a medical diagnostics laboratory at the Mayo Clinic.

Therefore, one skilled in the art would recognize that Ahlquist discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraphs 55-56.

'781 Patent, Claim 1, Second Limitation	**Ahlquist in view of De Luca and Olson**
b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample;	**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. *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

Ahlquist discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to Ahlquist, 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 in independent claim 1. The rest of the stool in the plastic bucket disclosed by Ahlquist corresponds to the 'remaining portion' of the fecal sample in independent claim 1.

Although Ahlquist utilizes the guaiac tests for detection of blood proteins, Ahlquist states that fecal immunochemical blood tests provide improved detection of adenomas compared to guaiac tests. Thus, a person of ordinary skill in the art reading Ahlquist would be motivated to substitute the guaiac tests of Ahlquist with means to perform fecal immunochemical blood tests because an improvement in detection would be achieved. Ex. 1014 at paragraph 68.

De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are fecal immunochemical blood tests indicated by Ahlquist to provide improved adenoma detection compared to guaiac tests.

Further, De Luca teaches that fecal samples can be removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a buffer solution and fecal sample and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca for performing fecal immunochemical blood tests instead of the guaiac tests of Ahlquist with the requisite expectation of success. Therefore, one skilled in the art would recognize that Ahlquist in view of De Luca discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 68.

'781 Patent, Claim 1, Third Limitation	**Ahlquist in view of De Luca and Olson**
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or	**Ahlquist** discloses "[p]atients collected 3 stools by using plastic buckets mounted to the toilet seat. Promptly after each individual

degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	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. *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for

	containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

As discussed *supra*, Ahlquist discloses removing a portion of the fecal sample by smearing stool onto the guaiac-based Hemoccult and HemoccultSensa cards. Ahlquist states that fecal immunochemical blood tests provide improved detection of adenomas compared to guaiac tests. Ex. 1004 at page 9, second full paragraph. Thus, a person of ordinary skill in the art reading Ahlquist would be motivated to substitute the guaiac tests of Ahlquist with means to perform fecal immunochemical blood tests because an improvement in detection would be achieved. Ex. 1014 at paragraph 76.

De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are fecal immunochemical blood tests indicated by Ahlquist to provide improved adenoma detection compared to guaiac tests.

Further, De Luca teaches that fecal samples can be removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a buffer solution and fecal sample and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca in order to perform a fecal immunochemical blood test instead of the guaiac tests of Ahlquist. Based on De Luca, a person of ordinary skill in the art

would combine the removed portion of the fecal sample in the separate sealable container with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Ex. 1014 at paragraph 76. Further, De Luca teaches that the extraction tube is sealed using a top cap, rendering the extraction tube to be a "separate sealable container." Therefore, one skilled in the art would recognize that Ahlquist in view of De Luca discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Fourth Limitation	**Ahlquist in view of De Luca and Olson**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**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 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." *Id.* 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." *Id.*

	Olson discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Ahlquist discloses removing a portion of the fecal sample by smearing stool onto the guaiac-based Hemoccult and HemoccultSensa cards, corresponding to the "removed portion" of the fecal sample. After removing this portion, Ahlquist discloses that the remaining portion of the fecal sample is "sealed in a bucket" and express-shipped to the testing facility along with the Hemoccult and HemoccultSensa cards.

Although Ahlquist does not teach addition of a stabilizing buffer to the remaining portion of the fecal sample, 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." Ex. 1004 at page 9, third full paragraph (citations omitted). To this end, 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." *Id.* Thus, a person of ordinary skill in the art reading Ahlquist would be motivated to add a preservative as a stabilizing buffer to the stool at the time of defecation in order to prevent degradation of nucleic acids. Ex. 1014 at paragraphs 85-86.

Olson discloses that addition of a stabilizing buffer (Exact buffer A) to stool samples provided a significant increase in recovery of DNA compared with samples incubated without buffer. This addition of a stabilizing buffer to DNA led to increased assay sensitivity.

Thus, a person of ordinary skill in the art would have been motivated to combine the remaining portion of the fecal sample with the stabilizing buffer taught by Olson instead of omitting the buffer as was done in Ahlquist. Olson discloses that DNA assays are greatly improved with addition of stabilizing buffer and Ahlquist provides express motivation to make this modification with the requisite expectation of success. Therefore, one skilled in the art would recognize that Ahlquist in view of Olson discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. *Id.*

In summary, Ahlquist in view of De Luca and Olson disclose the limitations of claim 1 of the '781 Patent. Consequently, claim 1 is obvious over Ahlquist in view of De Luca and Olson.

8. O'Neill in view of De Luca and Olson Renders Claim 1 Obvious

'781 Patent, Claim 1, Preamble	O'Neill in view of De Luca and Olson
1. A method of processing a freshly-collected fecal sample without freezing, the method comprising:	**O'Neill** discloses "a fixed shape container for collecting faecal stools which has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel." Ex. 1005 at page 7, lines 5-11. O'Neill discloses that the container "can serve both as the receptacle into which the stool is excreted and as the container in which the stool is washed. So the stool does not need to be transferred to a different washing container, which avoids damage to the stool surface caused by such a transfer. Such damage would interfere with the accuracy of e.g. screening tests which may be performed on the stool. If the stool is excreted into the container at home and then transported to e.g. a clinic for washing, the container has the further advantage of holding the stool reasonably securely in the channel so that transportation damage to the stool surface is reduced or avoided." *Id.* at page 7, line 34-page 8, line 11. O'Neill discloses that the container can be placed in an apparatus and that 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." *Id.* at page 6, lines 14-17.

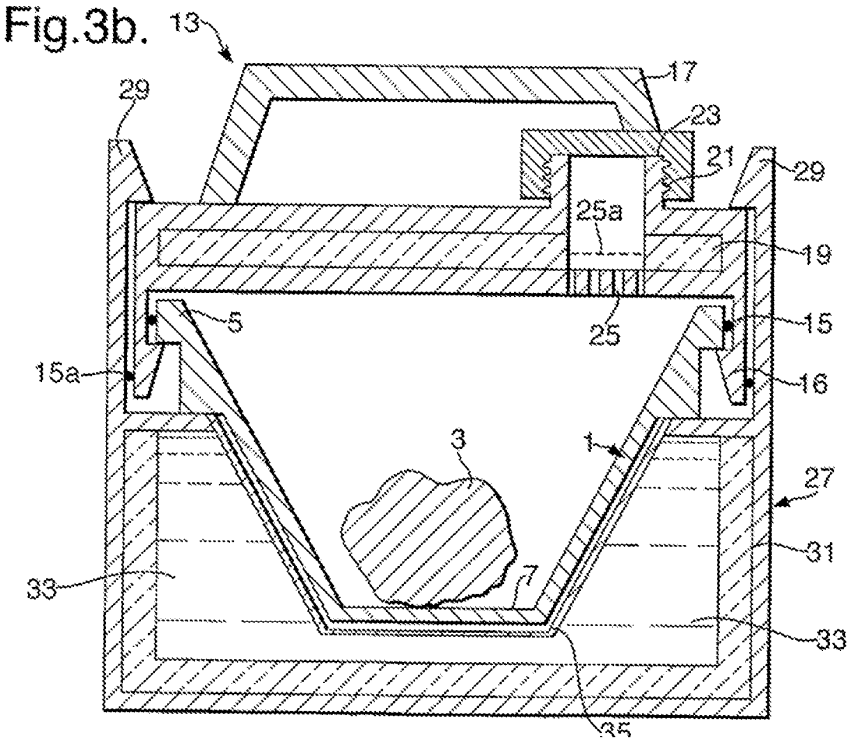
As explained in Section IV. *supra*, the preamble of claim 1 is not a limitation. However, even if the preamble of claim 1 was determined to be a limitation, O'Neill discloses this limitation because O'Neill teaches collection of stool (a fecal sample) into a container. Further, although O'Neill suggests that the stools can be cooled to a temperature "below its gel freezing point," this is simply one embodiment of the disclosure. Moreover, the temperature range of cooling the stool includes temperatures as high as 15°C (59°F). A person of ordinary skill in the art would understand that collection of a fecal sample at a temperature of 15°C (59°F) corresponds to processing "without freezing." Ex. 1014 at paragraph 49.

Accordingly, a person of ordinary skill in the art would recognize O'Neill discloses the method of processing a "freshly-collected" fecal sample "without freezing" specified by the preamble. *Id.*

'781 Patent, Claim 1, First Limitation	**O'Neill in view of De Luca and Olson**
a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel;	**O'Neill** discloses "a fixed shape container for collecting faecal stools which has an upper receiving mouth and a lower stool collection channel, the internal walls of the container narrowing from the mouth to the channel so that when a stool is deposited through the mouth the stool is encouraged to lie lengthways along the channel." Ex. 1005 at page 7, lines 5-11. O'Neill discloses that the container "can serve both as the receptacle into which the stool is excreted and as the container in which the stool is washed. … If

	the stool is excreted into the container at home and then transported to e.g. a clinic for washing, the container has the further advantage of holding the stool reasonably securely in the channel so that transportation damage to the stool surface is reduced or avoided." *Id.* at page 7, line 34- page 8, line 11. O'Neill discloses that "preferably the container is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein. The seal may be achieved by means of a lid or cover (e.g. having a snap-fit or screw fitting) for the mouth." *Id.* at page 9, lines 28-33.

O'Neill discloses collecting a fecal sample from a human subject as it teaches that human patients collected stools (a fecal sample) into a container that is positioned over a toilet. The container is a collection vessel and O'Neill teaches that the container is "the receptacle into which the stool is excreted." Figure 3b of O'Neill is presented below, showing the receptacle (1) of the container that includes an excreted stool (3).



O'Neill discloses that "the container is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein. The seal may be achieved by means of a lid or cover (e.g. having a snap-fit or screw fitting) for the mouth." This indicates that the container of O'Neill is a sealable collection vessel. Figure 3b of O'Neill shows the lid (13) that seals the container of the receptacle (1).

Further, O'Neill discloses that "the stool is excreted into the container at home and then transported to e.g. a clinic for washing." This indicates that the stool can be collected in a toilet that is not located at a testing facility.

Therefore, one skilled in the art would recognize that O'Neill discloses all of the requirements for the collecting step contained in the first limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 58.

'781 Patent, Claim 1, Second Limitation	**O'Neill in view of De Luca and Olson**
b) removing a portion of the fecal sample to a separate sealable container to produce a	**O'Neill** discloses "a method and apparatus for removing cellular material from faecal stools

removed portion and a remaining portion of the fecal sample;	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." *Id.* 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." *Id.* 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." *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

O'Neill discloses removing a portion of the fecal sample to produce a removed portion and a remaining portion of the fecal sample. According to O'Neill, cellular material is removed from a fecal sample. This cellular material corresponds to the 'removed portion' of the fecal sample in independent claim 1. The rest of the stool in the container disclosed by O'Neill corresponds to the 'remaining portion' of the fecal sample in independent claim 1.

O'Neill discloses that the cellular material is removed from the fecal sample via a wash liquid and teaches that the removed portion is placed into sample receptacles for subsequent analysis of DNA, RNA and proteins. In particular, O'Neill discloses that "[w]hen the wash programme ends, the sample rack is pivoted about pivot 47 to bring axis 41 into a vertical position (see Fig. 5). Almost all of wash liquid 44 is then decanted out of receptacle 1 via port 21, foraminous screen 25 substantially preventing larger food residues or other broken off pieces from the stool exiting with the liquid. Coarse sieve 25a removes smaller pieces from the liquid. Finer downstream sieves (not shown) further limit the maximum particle size in the liquid. The liquid is then be divided into standard volumes and received into a plurality of removable (e.g. clip-on) sample receptacles (not shown). The sample receptacles may be a strip of tubes each of e.g. 0.1-0.5 ml volume as can be removed and clipped into a standard microplate format used for clinical analyses. Alternatively the sample receptacles may be individual tubes each of e.g. 1-2 ml volume. The sample rack is then returned to its horizontal position and receptacle 1 is removed and safely disposed of." Ex. 1005 at page 14, lines 18-35. A person of ordinary skill in the art would understand that the individual tubes as described by O'Neill would include separate, sealable containers such as Eppendorf tubes that are used in the art. Ex. 1014 at paragraph 69.

Moreover, De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform assays, such as fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a fecal sample with buffer solution and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the fecal cellular material/wash liquids obtained by O'Neill with the requisite expectation of success. Therefore, one skilled in the art would recognize that O'Neill in view of De Luca discloses all of the requirements for the removing step contained in the second limitation of claim 1 of the '781 Patent. Ex. 1014 at paragraph 70.

'781 Patent, Claim 1, Third Limitation	**O'Neill in view of De Luca and Olson**
c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container; and	**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." *Id.* 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." *Id.* 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." *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of

	the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032].

As discussed *supra*, O'Neill discloses removing a portion of the fecal sample by removing cellular material from the stool, for instance with a "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." Ex. 1005 at page 13, lines 30-34. O'Neill teaches that the removed portion is placed into sample receptacles for subsequent analysis of DNA, RNA and proteins. Ex. 1005 at page 1, lines 15-20. Thus, a person of ordinary skill in the art reading O'Neill would be motivated to stabilize the removed cellular material so that it could provide a more accurate assessment for patients. Ex. 1014 at paragraph 77.

De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are used for detection of blood proteins such as hemoglobin for use in fecal immunochemical blood tests. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. The extraction tube of De Luca includes a container body for containing a buffer solution and fecal sample and the container is sealed using a cap.

Thus, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to place the removed cellular material from the fecal sample of O'Neill. Based on De Luca, a person of ordinary skill in the art would combine the removed portion of the fecal sample in the separate sealable container with a buffer in the

extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Ex. 1014 at paragraph 77. Further, De Luca teaches that the extraction tube is sealed using a top cap, rendering the extraction tube to be a "separate sealable container." Therefore, one skilled in the art would recognize that O'Neill in view of De Luca discloses all of the requirements for the combining step contained in the third limitation of claim 1 of the '781 Patent. *Id.*

'781 Patent, Claim 1, Fourth Limitation	**O'Neill in view of De Luca and Olson**
d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel.	**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." *Id.* 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." *Id.* at page 6, line 31 – page 7, line 4. Finally, O'Neill discloses that "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)." *Id.* at page 15, lines 1-4. **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of

	stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, O'Neill discloses removing a portion of the fecal sample by removing cellular material from the stool. After removing this portion, O'Neill discloses that the remaining portion of the fecal sample is still in the container. For instance, O'Neill states that a typical total stool has a mass of 100-500 grams and that the removed cellular material has a mass of about 50-1000 mg. Ex. 1005 at page 15, lines 1-4. Thus, a person of ordinary skill in the art would understand that O'Neill teaches a remaining portion of the stool of at least 99 grams. Ex. 1014 at paragraph 87. Although O'Neill does not teach addition of a stabilizing buffer to the remaining portion of the fecal sample, a person of ordinary skill in the art would be motivated to stabilize the remaining stool sample so that subsequent analyses could be made. Ex. 1014 at paragraphs 87-88.

Olson discloses that addition of a stabilizing buffer (Exact buffer A) to stool samples provided a significant increase in recovery of DNA compared with samples incubated without buffer. This addition of a stabilizing buffer to DNA led to increased assay sensitivity.

Thus, a person of ordinary skill in the art would have been motivated to combine the remaining portion of the fecal sample with the stabilizing buffer taught by Olson instead of omitting the buffer as was done in O'Neill. Olson discloses that DNA assays are greatly improved with addition of stabilizing buffer and O'Neill provides express motivation to make this modification with the requisite expectation of success. Therefore, one skilled in the art would recognize that O'Neill in view of Olson discloses all of the requirements for the combining step contained in the fourth limitation of claim 1 of the '781 Patent. *Id.*

In summary, O'Neill in view of De Luca and Olson disclose the limitations of claim 1 of the '781 Patent. Consequently, claim 1 is obvious over O'Neill in view of De Luca and Olson.

B. Dependent Claim 2

1. Lapidus '178 Anticipates Claim 2

'781 Patent, Claim 2	Lapidus '178
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	Lapidus '178 describes that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1002 at column 7, lines 63-64.

As discussed above, Lapidus '178 describes the elements of claim 1 and further describes that the receptacle is "small enough to be transported to a testing facility." The testing facility according to the broadest reasonable interpretation of the claims consistent with the specification includes a testing facility (e.g., a medical diagnostics laboratory) that would perform the diagnostic assays on the stool sample. Further, Lapidus '178 describes transportation to a testing facility and a person of ordinary skill in the art would understand that such transportation would include the portions as provided in independent claim 1. Ex. 1014 at paragraph 98.

Lapidus '178 therefore describes the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are anticipated for the reasons explained above for claim 1, claim 2 is also anticipated by Lapidus '178.

2. Lapidus '650 Anticipates Claim 2

'781 Patent, Claim 2	Lapidus '650
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal	Lapidus '650 describes that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1003 at column 7, lines 24-26.

sample and said stabilizing buffer to a medical diagnostics laboratory.	

As discussed above, Lapidus '650 describes the elements of claim 1 and further describes that the receptacle is "small enough to be transported to a testing facility." The testing facility according to the broadest reasonable interpretation of the claims consistent with the specification includes a testing facility (e.g., a medical diagnostics laboratory) that would perform the diagnostic assays on the stool sample. Further, Lapidus '650 describes transportation to a testing facility and a person of ordinary skill in the art would understand that such transportation would include the portions as provided in independent claim 1. Ex. 1014 at paragraph 98.

Lapidus '650 therefore describes the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are anticipated for the reasons explained above for claim 1, claim 2 is also anticipated by Lapidus '650.

3. Lapidus '178 Renders Claim 2 Obvious

'781 Patent, Claim 2	Lapidus '178
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	**Lapidus '178** discloses that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1002 at column 7, lines 63-64.

In the alternative to the assertion that Lapidus '178 anticipates claim 2, Lapidus '178 further renders claim 2 obvious.

As discussed above, Lapidus '178 discloses the elements of claim 1 and further describes that the receptacle is "small enough to be transported to a testing facility." The testing facility according to the broadest reasonable interpretation of the claims consistent with the specification includes a testing facility (e.g., a medical diagnostics laboratory) that would perform the

diagnostic assays on the stool sample. It would have been known to the person of ordinary skill in the art would understand that transportation according to Lapidus '178 would include delivery of the portions as provided in independent claim 1. Ex. 1014 at paragraph 99.

Lapidus '178 therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 is also obvious over Lapidus '178. Accordingly, Lapidus '178 renders claim 2 obvious.

4. Lapidus '650 Renders Claim 2 Obvious

'781 Patent, Claim 2	Lapidus '650
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	**Lapidus '650** describes that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1003 at column 7, lines 24-26.

In the alternative to the assertion that Lapidus '650 anticipates claim 2, Lapidus '650 further renders claim 2 obvious.

As discussed above, Lapidus '650 discloses the elements of claim 1 and further describes that the receptacle is "small enough to be transported to a testing facility." The testing facility according to the broadest reasonable interpretation of the claims consistent with the specification includes a testing facility (e.g., a medical diagnostics laboratory) that would perform the diagnostic assays on the stool sample. It would have been known to the person of ordinary skill in the art would understand that transportation according to Lapidus '650 would include delivery of the portions as provided in independent claim 1. Ex. 1014 at paragraph 98.

Lapidus '650 therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 is also obvious over Lapidus '650. Accordingly, Lapidus '650 renders claim 2 obvious.

5. Lapidus '178 in view of De Luca and Olson Renders Claim 2 Obvious

'781 Patent, Claim 2	Lapidus '178 in view of De Luca and Olson
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	**Lapidus '178** discloses that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1002 at column 7, lines 63-64.

As discussed *supra*, Lapidus '178 in view of De Luca and Olson disclose the limitations of claim 1, namely a sealable container containing the removed portion of the fecal sample and buffer and a sealable collection vessel containing the remaining portion of the fecal sample and stabilizing buffer.

Further, Lapidus '178 discloses delivering fecal sample-containing items to a clinic for analysis. A person of ordinary skill in the art would understand that a clinic in this context would contain a medical diagnostics laboratory. Ex. 1014 at paragraphs 98-99.

Lapidus '178 in view of De Luca and Olson therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 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 2 Obvious

'781 Patent, Claim 2	Lapidus '650 in view of De Luca and Olson
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal	**Lapidus '650** describes that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1003 at column 7, lines 24-26.

sample and said stabilizing buffer to a medical diagnostics laboratory.	

As discussed *supra*, Lapidus '650 in view of De Luca and Olson disclose the limitations of claim 1, namely a sealable container containing the removed portion of the fecal sample and buffer and a sealable collection vessel containing the remaining portion of the fecal sample and stabilizing buffer.

Further, Lapidus '650 discloses delivering fecal sample-containing items to a testing facility (e.g., a medical diagnostics laboratory) for analysis. A person of ordinary skill in the art would understand that a testing facility in this context would contain a medical diagnostics laboratory. Ex. 1014 at paragraphs 98-99.

Lapidus '650 in view of De Luca and Olson therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 is also obvious over Lapidus '650 in view of De Luca and Olson.

7. Ahlquist in view of De Luca and Olson Renders Claim 2 Obvious

'781 Patent, Claim 2	**Ahlquist in view of De Luca and Olson**
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	**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.

As discussed *supra*, Ahlquist in view of De Luca and Olson disclose the limitations of claim 1, namely a sealable container containing the removed portion of the fecal sample and buffer and a sealable collection vessel containing the remaining portion of the fecal sample and stabilizing buffer.

Further, Ahlquist discloses delivering fecal sample-containing items to a medical diagnostics laboratory. Ahlquist discloses that the items are express-shipped to the Mayo Clinic in Rochester, Minnesota. It is well known that the Mayo Clinic contains a medical diagnostics laboratory, and in fact Ahlquist further discloses diagnostic tests that are performed on the fecal samples that are mailed. Ex. 1014 at paragraph 100.

Ahlquist in view of De Luca and Olson therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 is also obvious over Ahlquist in view of De Luca and Olson.

8. O'Neill in view of De Luca and Olson Renders Claim 2 Obvious

'781 Patent, Claim 2	O'Neill in view of De Luca and Olson
2. The method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory.	**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." *Id.* at page 8, lines 7-11.

As discussed *supra*, O'Neill in view of De Luca and Olson disclose the limitations of claim 1, namely a sealable container containing the removed portion of the fecal sample and buffer and a sealable collection vessel containing the remaining portion of the fecal sample and stabilizing buffer.

Further, O'Neill discloses delivering fecal sample-containing items to a clinic for analysis. A person of ordinary skill in the art would understand that a clinic in this context would contain a medical diagnostics laboratory. Ex. 1014 at paragraph 101.

O'Neill in view of De Luca and Olson therefore discloses the additional limitations of claim 2 of the '781 Patent. Because the other limitations of claim 2 are obvious for the reasons explained above for claim 1, claim 2 is also obvious over O'Neill in view of De Luca and Olson.

C. Dependent Claim 3

1. Lapidus '178 Anticipates Claim 3

'781 Patent, Claim 3, Preamble	Lapidus '178
3. A method of processing a fecal sample, the method comprising:	Lapidus '178 describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '178 describes this limitation because Lapidus '178 describes processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing.

'781 Patent, Claim 3, First Limitation	Lapidus '178
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	Lapidus '178 describes obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent claim 1, Lapidus '178 describes each and every element of independent claim 1.

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;	

Lapidus '178 describes obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra*, one skilled in the art would recognize that Lapidus '178 describes 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	**Lapidus '178**
b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;	**Lapidus '178** describes 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 describes 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." *Id.* at column 3, lines 26-30. Lapidus '178 describes 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." *Id.* at column 9, lines 40-45.

Lapidus '178 describes 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. Further, Lapidus '178 describes 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 57-60.

Furthermore, Lapidus '178 describes 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 115.

Therefore, one skilled in the art would recognize that Lapidus '178 describes 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	**Lapidus '178**
c) extracting nucleic acid from the remaining portion of the fecal sample; and	**Lapidus '178** describes that "a whole stool may be used for diagnostic assays. The stool or portion of stool that is obtained contains sufficient material to allow subsequent diagnostic assays to be performed." Ex. 1002 at column 9, lines 26-29. Lapidus '178 describes 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 U.S. Pat. No. 5,670,325 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. 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." *Id.* at column 6, line 50 – column 7, line 2. Lapidus '178 describes "[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." *Id.* at column 9, line 62 – column 10, line 6.

Lapidus '178 describes extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, it is logical that Lapidus '178 describes a 'remaining portion' of the fecal sample that is left over after removal of a cross-sectional, circumferential surface, and/or layer of the stool -- logic dictates that. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '178 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 124-125. Further, as discussed *supra*, Lapidus '178 describes two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. See Ex. 1002 at column 3, lines 11-15.

Lapidus '178 contemplates that '[t]he ***stool*** or ***portion of stool*** that is obtained contains sufficient material to allow subsequent diagnostic assays to be performed." *Id.* at column 9, lines 27-29 (emphasis added). Such assays include those for the detection of sequences in selected

DNA molecules, detection of nucleic acid sequences, detection of mutant alleles, RFLP analysis, detection of p53 gene sequences, detection of MCC gene sequences, detection of APC gene sequences, and detection of DCC gene sequences, all of which are incorporated by reference into the specification of Lapidus '178. For instance, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '178 at column 6, line 60) provides that it relates to methods and compositions for detecting the presence or absence of a target DNA sequence, such as a mutation, within an identified region of a selected DNA molecule, such as a gene." Furthermore, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '178 at column 6, line 60) states that "[h]uman DNA specimens from SW480 and SW403 colon carcinoma cell lines and HL60 leukemia cell line were extracted by standard technique of proteinase K digestion and phenol/chloroform extraction (Ausubel, et al. Eds.: Current Protocols in Molecular Biology. Chapter 2, section 1 and 2. Greene Publishing Assoc. and Wiley-Interscience, New York, 1987)." A person of ordinary skill in the art understands that such assay involve extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '178 describes assays that comprise extracting nucleic acid in order to perform the evaluation.

Furthermore, Lapidus '178 describes that DNA or RNA can be isolated from the sample according to methods known in the art, including those incorporated by reference in Smith-Ravin, et al., *Gut*, 36: 81-86 (1995). According to Smith-Ravin et al., "DNA was extracted from both the centrifugation pellet and supernatant fractions, as well as from endoscopy washings, using a conventional phenol chloroform extraction method and was then purified on glass milk or spin columns. The purified DNA was amplified using mitochondrial primers and analysed for ras mutations using a non-radioactive, allele specific mismatch method." Accordingly, it is well known to the skilled artisan that 'isolation' of nucleic acid refers to an extraction of nucleic acid. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '178 describes an assay in which nucleic acid (e.g., DNA or RNA) is extracted.

Therefore, one skilled in the art would recognize that Lapidus '178 describes 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	Lapidus '178
d) testing the nucleic acid for an amount of a human nucleic acid.	**Lapidus '178** describes a protocol entitled "1. Method for Quantitation of Target and Reference Polynucleotides." Ex. 1002 at column 11, lines 19-20. Here, Lapidus '178 describes "[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." *Id.* at column 7, lines 21-27.

Lapidus '178 describes testing the nucleic acid for an amount of a human nucleic acid. Example 2 of Lapidus '178 describes 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 '178 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.*

Therefore, one skilled in the art would recognize that Lapidus '178 describes all of the requirements for the testing step contained in the fourth limitation of claim 3 of the '781 Patent. *Id.*

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

2. Lapidus '650 Anticipates Claim 3

'781 Patent, Claim 3, Preamble	Lapidus '650
3. A method of processing a fecal sample, the method comprising:	**Lapidus '650** describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '650 describes this limitation because Lapidus '650 describes processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing.

'781 Patent, Claim 3, First Limitation	Lapidus '650
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;	Lapidus '650 describes obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent claim 1, Lapidus '650 describes each and every element of independent claim 1.

Lapidus '650 describes obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra*, one skilled in the art would recognize that Lapidus '650 describes 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	**Lapidus '650**
b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;	**Lapidus '650** discloses that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 8, lines 5-7. Lapidus '650 discloses "[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer". *Id.* at column 6, lines 1-11. Lapidus '650 describes 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." *Id.* at column 3, lines 4-9. Lapidus '650 describes 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." *Id.* at column 8, lines 13-18.

Lapidus '650 describes 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 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. Further, Lapidus '650 describes 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.

Furthermore, Lapidus '650 describes 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 115.

Therefore, one skilled in the art would recognize that Lapidus '650 describes 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	**Lapidus '650**
c) extracting nucleic acid from the remaining portion of the fecal sample; and	Lapidus '650 describes 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 describes 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." *Id.* at column 6, lines 6-28. Lapidus '650 describes "[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." *Id.* at column 8, lines 39-50.

Lapidus '650 describes extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, it is logical that Lapidus '650 describes a 'remaining portion' of the fecal sample that is left over after removal of a cross-sectional, circumferential surface, and/or layer of the stool -- logic dictates that. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 describes the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 124-125.

Lapidus '650 contemplates assays include those for the detection of sequences in selected DNA molecules, detection of nucleic acid sequences, detection of mutant alleles, RFLP analysis, detection of p53 gene sequences, detection of MCC gene sequences, detection of APC gene sequences, and detection of DCC gene sequences, all of which are incorporated by reference into the specification of Lapidus '650. For instance, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '650 at column 6, line 17) provides that it relates to

methods and compositions for detecting the presence or absence of a target DNA sequence, such as a mutation, within an identified region of a selected DNA molecule, such as a gene." Furthermore, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '650 at column 6, line 17) states that "[h]uman DNA specimens from SW480 and SW403 colon carcinoma cell lines and HL60 leukemia cell line were extracted by standard technique of proteinase K digestion and phenol/chloroform extraction (Ausubel, et al. Eds.: Current Protocols in Molecular Biology. Chapter 2, section 1 and 2. Greene Publishing Assoc. and Wiley-Interscience, New York, 1987)." A person of ordinary skill in the art understands that such assay involve extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '650 describes assays that comprise extracting nucleic acid in order to perform the evaluation.

Furthermore, Lapidus '650 describes that DNA or RNA can be isolated from the sample according to methods known in the art, including those incorporated by reference in Smith-Ravin, et al., *Gut*, 36: 81-86 (1995). According to Smith-Ravin et al., "DNA was extracted from both the centrifugation pellet and supernatant fractions, as well as from endoscopy washings, using a conventional phenol chloroform extraction method and was then purified on glass milk or spin columns. The purified DNA was amplified using mitochondrial primers and analysed for ras mutations using a non-radioactive, allele specific mismatch method." Accordingly, it is well known to the skilled artisan that 'isolation' of nucleic acid refers to an extraction of nucleic acid. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '650 describes an assay in which nucleic acid (e.g., DNA or RNA) is extracted.

Therefore, one skilled in the art would recognize that Lapidus '650 describes 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	**Lapidus '650**
d) testing the nucleic acid for an amount of a human nucleic acid.	Lapidus '650 describes a protocol entitled "1. Method for Quantitation of Target and Reference Polynucleotides." Ex. 1003 at column 9, lines 63-65. Here, Lapidus '650 describes "[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." *Id.* at column 9, lines 66 – column 10, line 5.

Lapidus '650 describes testing the nucleic acid for an amount of a human nucleic acid. Example 2 of Lapidus '650 describes 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.*

Therefore, one skilled in the art would recognize that Lapidus '650 describes all of the requirements for the testing step contained in the fourth limitation of claim 3 of the '781 Patent. *Id.*

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

3. Lapidus '178 Renders Claim 3 Obvious

'781 Patent, Claim 3, Preamble	**Lapidus '178**
3. A method of processing a fecal sample, the method comprising:	**Lapidus '178** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at

	column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

In the alternative to the assertion that Lapidus '178 anticipates claim 3, Lapidus '178 further renders claim 3 obvious.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '178 discloses this limitation because Lapidus '178 discloses processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing. Accordingly, Lapidus '178 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, First Limitation	**Lapidus '178**
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;	**Lapidus '178** discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent claim 1, Lapidus '178 describes each and every element of independent claim 1.

Lapidus '178 discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra*, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements

for the obtaining step contained in the first limitation of claim 3 of the '781 Patent. x. 1014 at paragraphs 112-113. Accordingly, Lapidus '178 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Second Limitation	**Lapidus '178**
b) testing the removed portion of the fecal sample for an amount of a blood protein present 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." *Id.* 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." *Id.* at column 9, lines 40-45.

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 115.

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* Accordingly, Lapidus '178 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Third Limitation	**Lapidus '178**
c) extracting nucleic acid from the remaining portion of the fecal sample; and	**Lapidus '178** discloses that "a whole stool may be used for diagnostic assays. The stool or portion of stool that is obtained contains sufficient material to allow subsequent diagnostic assays to be performed." Ex. 1002 at column 9, lines 26-29. Lapidus '178 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 U.S. Pat. No. 5,670,325 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. 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." *Id.* at column 6, line 50 – column 7, line 2. 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." *Id.* at column 9, line 62 – column 10, line 6.

Lapidus '178 discloses extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, it is logical that Lapidus '178 discloses a 'remaining portion' of the fecal sample that is left over after removal of a cross-sectional, circumferential surface, and/or layer of the stool. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '178 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 124-125. Further, as discussed *supra*, Lapidus '178 discloses two different parts of fecal material – the 'removed portion' ***sample of stool*** and also the 'remaining portion' of ***the stool***. See Ex. 1002 at column 3, lines 11-15.

Lapidus '178 contemplates that '[t]he ***stool*** or ***portion of stool*** that is obtained contains sufficient material to allow subsequent diagnostic assays to be performed." *Id.* at column 9, lines 27-29 (emphasis added). Such assays include those for the detection of sequences in selected DNA molecules, detection of nucleic acid sequences, detection of mutant alleles, RFLP analysis, detection of p53 gene sequences, detection of MCC gene sequences, detection of APC gene sequences, and detection of DCC gene sequences, all of which are incorporated by reference into the specification of Lapidus '178. For instance, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '178 at column 6, line 60) provides that it relates to methods and compositions for detecting the presence or absence of a target DNA sequence, such as a mutation, within an identified region of a selected DNA molecule, such as a gene." A person of ordinary skill in the art understands that such assay involve extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '178 discloses assays comprise extracting nucleic acid in order to perform the evaluation.

Furthermore, Lapidus '178 discloses that DNA or RNA that can be isolated from the sample according to methods known in the art. It is well known to the skilled artisan that 'isolation' of nucleic acid refers to an extraction of nucleic acid. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '178 discloses an assay in which nucleic acid (e.g., DNA or RNA) is extracted.

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the extracting step contained in the third limitation of claim 3 of the '781 Patent. *Id.* Accordingly, Lapidus '178 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Fourth Limitation	**Lapidus '178**
d) testing the nucleic acid for an amount of a human nucleic acid.	**Lapidus '178** discloses a protocol entitled "1. Method for Quantitation of Target and Reference Polynucleotides." Ex. 1002 at column 11, lines 19-20. Here, Lapidus '178 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." *Id.* at column 7, lines 21-27.

Lapidus '178 discloses testing the nucleic acid for an amount of a human nucleic acid. Example 2 of Lapidus '178 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 '178 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.*

Therefore, one skilled in the art would recognize that Lapidus '178 discloses all of the requirements for the testing step contained in the fourth limitation of claim 3 of the '781 Patent. *Id.*

Lapidus '178 therefore discloses the additional limitations of claim 3 of the '781 Patent. Because the other limitations of claim 3 are obvious the reasons explained above for claim 1, claim 3 is also obvious over Lapidus '178. Accordingly, Lapidus '178 renders this portion of claim 3 obvious.

4. Lapidus '650 Renders Claim 3 Obvious

'781 Patent, Claim 3, Preamble	Lapidus '650
3. A method of processing a fecal sample, the method comprising:	**Lapidus '650** describes processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26.

In the alternative to the assertion that Lapidus '650 anticipates claim 3, Lapidus '650 further renders claim 3 obvious.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '650 discloses this limitation because Lapidus '650 discloses processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing. Accordingly, Lapidus '650 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, First Limitation	Lapidus '650
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	**Lapidus '650** discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent

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;	claim 1, Lapidus '650 describes each and every element of independent claim 1.

Lapidus '650 discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra*, one skilled in the art would recognize that Lapidus '650 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. Accordingly, Lapidus '650 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Second Limitation	**Lapidus '650**
b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion;	**Lapidus '650** discloses that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 8, lines 5-7. Lapidus '650 discloses "[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation

	such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer". *Id.* at column 6, lines 1-11. 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." *Id.* 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." *Id.* at column 8, lines 13-18.

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.

Furthermore, 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." 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 115.

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the testing step contained in the second limitation of claim 3 of the '781 Patent. *Id.* Accordingly, Lapidus '650 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Third Limitation	**Lapidus '650**
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." *Id.* 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." *Id.* at column 8, lines 39-50.

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, circumferential surface, and/or layer of the stool -- logic dictates that. *In re Johnson*, 558 F.2d at 1019. A person of ordinary skill in the art would recognize and understand that Lapidus '650 discloses the 'remaining portion' according to independent claim 1. Ex. 1014 at paragraphs 124-125.

Lapidus '650 contemplates assays include those for the detection of sequences in selected DNA molecules, detection of nucleic acid sequences, detection of mutant alleles, RFLP analysis, detection of p53 gene sequences, detection of MCC gene sequences, detection of APC gene sequences, and detection of DCC gene sequences, all of which are incorporated by reference into the specification of Lapidus '650. For instance, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '650 at column 6, line 17) provides that it relates to methods and compositions for detecting the presence or absence of a target DNA sequence, such as a mutation, within an identified region of a selected DNA molecule, such as a gene." Furthermore, U.S. Patent No. 5,137,806 (Ex. 1012; incorporated by reference into Lapidus '650 at column 6, line 17) states that "[h]uman DNA specimens from SW480 and SW403 colon carcinoma cell lines and HL60 leukemia cell line were extracted by standard technique of proteinase K digestion and phenol/chloroform extraction (Ausubel, et al. Eds.: Current Protocols in Molecular Biology. Chapter 2, section 1 and 2. Greene Publishing Assoc. and Wiley-Interscience, New York, 1987)." A person of ordinary skill in the art understands that such assay involve extraction of nucleic acid in order to perform the assay. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '650 discloses assays that comprise extracting nucleic acid in order to perform the evaluation.

Furthermore, Lapidus '650 discloses that DNA or RNA can be isolated from the sample according to methods known in the art, including those incorporated by reference in Smith-Ravin, et al., *Gut*, 36: 81-86 (1995). According to Smith-Ravin et al., "DNA was extracted from both the centrifugation pellet and supernatant fractions, as well as from endoscopy washings, using a conventional phenol chloroform extraction method and was then purified on glass milk or spin columns. The purified DNA was amplified using mitochondrial primers and analysed for ras mutations using a non-radioactive, allele specific mismatch method." Accordingly, it is well known to the skilled artisan that 'isolation' of nucleic acid refers to an extraction of nucleic acid. Ex. 1014 at paragraphs 124-125. Thus, Lapidus '650 discloses an assay in which nucleic acid (e.g., DNA or RNA) is extracted.

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the extracting step contained in the third limitation of claim 3 of the '781 Patent. *Id.* Accordingly, Lapidus '650 renders this portion of claim 3 obvious.

'781 Patent, Claim 3, Fourth Limitation	Lapidus '650
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." *Id.* at column 9, lines 66 – column 10, line 5.

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 disclosed assays. A person of ordinary skill in the art would understand that the disclosed nucleic acids are human nucleic acid. *Id.*

Therefore, one skilled in the art would recognize that Lapidus '650 discloses all of the requirements for the testing step contained in the fourth limitation of claim 3 of the '781 Patent. *Id.*

Lapidus '650 therefore discloses the additional limitations of claim 3 of the '781 Patent. Because the other limitations of claim 3 are obvious the reasons explained above for claim 1, claim 3 is also obvious over Lapidus '650. Accordingly, Lapidus '650 renders this portion of claim 3 obvious.

5. Lapidus '178 in view of De Luca and Olson Renders Claim 3 Obvious

'781 Patent, Claim 3, Preamble	Lapidus '178 in view of De Luca and Olson
3. A method of processing a fecal sample, the method comprising:	**Lapidus '178** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1002 at column 7, lines 58-62. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 63-64.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '178 discloses this limitation because Lapidus '178 discloses processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing. Furthermore, as discussed *supra*, Lapidus '178 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	Lapidus '178 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,	**Lapidus '178** discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent claim 1, Lapidus '178 describes each and every element of independent claim 1. **De Luca** discloses "an extraction and sampling device for collection of faeces

the pair of portions obtained by the method of claim 1;	samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032]. **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that

	"[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '178 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*, Lapidus '178 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 Lapidus '178 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	Lapidus '178 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;	**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." *Id.* 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." *Id.* 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 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. *Id.* 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." *Id.* at [0048].

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 115.

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 paragraph 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 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 118.

'781 Patent, Claim 3, Third Limitation	**Lapidus '178 in view of De Luca and Olson**
c) extracting nucleic acid from the remaining portion of the fecal sample; and	**Lapidus '178** discloses that "a whole stool may be used for diagnostic assays. The stool or portion of stool that is obtained contains sufficient material to allow subsequent diagnostic assays to be performed." Ex. 1002 at column 9, lines 26-29. Lapidus '178 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 U.S. Pat. No. 5,670,325 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. 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." *Id.* at column 6, line 50 – column 7, line 2. 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." *Id.* at column 9, line 62 – column 10, line 6. **Olson** discloses development of "a multitarget, fecal DNA screening assay that

	detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

Lapidus '178 discloses extracting nucleic acid from the remaining portion of the fecal sample. As discussed *supra*, it is logical that Lapidus '178 discloses a 'remaining portion' of the fecal sample that is left over after removal of a cross-sectional, circumferential surface, and/or

layer of the stool. Lapidus '178 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 '178 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 '178 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 '178 in view of De Luca and Olson**
d) testing the nucleic acid for an amount of a human nucleic acid.	**Lapidus '178** discloses a protocol entitled "1. Method for Quantitation of Target and Reference Polynucleotides." Ex. 1002 at column 11, lines 19-20. Here, Lapidus '178 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." *Id.* at column 7, lines 21-27.

	Olson discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

Lapidus '178 discloses testing the nucleic acid for an amount of a human nucleic acid. Example 2 of Lapidus '178 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 '178 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 '178 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 '178 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 '178 in view of De Luca and Olson.

6. Lapidus '650 in view of De Luca and Olson Renders Claim 3 Obvious

'781 Patent, Claim 3, Preamble	**Lapidus '650 in view of De Luca and Olson**
3. A method of processing a fecal sample, the method comprising:	**Lapidus '650** discloses processing of "a stool voided by a patient" in Example 1, entitled "Preparation of a Stool Sample." Ex. 1003 at column 7, lines 12-17. Further, "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility" for processing. *Id.* at column 7, lines 24-26.

Assuming, without conceding, that the preamble of claim 3 is a limitation, Lapidus '650 discloses this limitation because Lapidus '650 discloses processing a stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility for processing. Furthermore, as discussed *supra*, Lapidus '650 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	**Lapidus '650 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;	**Lapidus '650** discloses obtaining a pair of portions of a fecal sample collected from a human subject, wherein the pair of portions are obtained by the method of claim 1. As discussed *supra* with respect to independent claim 1, Lapidus '650 describes each and every element of independent claim 1. **De Luca** discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for

	containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032]. **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one

	exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '650 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*, Lapidus '650 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 Lapidus '650 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	**Lapidus '650 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;	**Lapidus '650** discloses that "[o]nce obtained, the cross-sectional stool sample is homogenized in an appropriate buffer, such as phosphate buffered saline." *Id.* at column 8, lines 5-7. Lapidus '650 discloses "[o]nce a cross-sectional stool sample is obtained, it may be homogenized by known methods to distribute cells and cellular debris throughout the sample. An assay then is performed on the homogenate, or an extract of the homogenate, to detect the presence of cells and/or cellular

	debris in the sample. The assay may be any one or a combination of histological cellular assays, antibody based immunoassays (or other formats) designed to detect the presence of a molecule characteristic of transformation such as a protein, or DNA-based assays for detecting mutations or genetic characteristics indicative of colorectal cancer". *Id.* at column 6, lines 1-11. 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." *Id.* 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." *Id.* 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. *Id.* 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." *Id.* at [0048].

Lapidus '650 discloses removing a portion of the fecal sample to produce a removed portion. As discussed *supra*, the 'removed portion' is a cross-sectional portion and Lapidus '650 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 '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." 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 115.

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 paragraph 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 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." *Id.* 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." *Id.* 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

	recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

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." *Id.* 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)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

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 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.

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. *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015].

	Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032]. 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." *Id.* 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." *Id.* **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments

	associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, 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.' Alquist 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." *Id.* 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)." *Id.* 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. *Id.*

	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." *Id.* at [0048].

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 "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 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 Hemoccult and HemoccultSensa 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." *Id.* 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." *Id.* **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer

	to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

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." *Id.* 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." *Id.* **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific

	mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

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." *Id.* 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." *Id.* 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." *Id.* 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." *Id.* 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, Helicobacter pylori and the like. Ex. 1006 at [0001]. De Luca discloses "an extraction and sampling tube for the collection of faeces samples that is practical for the patient and simple to use for the laboratory technician." *Id.* at [0015]. Further, De Luca discloses "[t]he extraction tube for collection of faeces samples, according to the invention, comprises a container body hollow on the inside for containing a buffer solution and faeces samples, a top cap applied to the top end of the container body and provided with a threaded small rod for sampling of faeces samples and a bottom cap applied removably to the bottom end of the container body. *Id.* at [0019]. De Luca discloses that on the extraction tube, "[a]n annular seal 55 is

	provided between the knurled part 44 and the threaded part 45 of the top cap." *Id.* at [0032]. **Olson** discloses development of "a multitarget, fecal DNA screening assay that detects the presence of gene-specific mutations and long DNA fragments associated with colorectal cancer (CRC)." Ex. 1007 at Abstract. Olson discloses that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer

	to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, 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." *Id.* 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. *Id.* 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." *Id.* at [0048].

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." *Id.* 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." *Id.* 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)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, 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." *Id.* 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." *Id.* 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)."

	Id. at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, 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." *Id.* at column 9, lines 47-49.

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." *Id.* 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." *Id.* 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." *Id.* 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." *Id.* 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)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '178 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." *Id.* 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)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, Lapidus '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." *Id.* at page 9, first full paragraph.

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." *Id.* 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

	exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

As discussed *supra*, 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).

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.

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).

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).

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).

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." *Id.* at column 6, line 58 – column 7, line 2.

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." *Id.* at column 6, lines 15-28.

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." *Id.* 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." *Id.* at column 6, lines 15-28.

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.

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." *Id.* 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.

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." *Id.* 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." *Id.* 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

	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. *Id.* 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." *Id.* at [0048].

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." *Id.* 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." *Id.* 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. *Id.* 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." *Id.* at [0048].

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)." *Id.* 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. *Id.* 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." *Id.* at [0048].

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." *Id.* 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. *Id.* 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." *Id.* at [0048].

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." *Id.* 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." *Id.* 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 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. *Id.* 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." *Id.* at [0048].

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". *Id.* 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." *Id.* 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." *Id.* 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. *Id.* 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." *Id.* at [0048].

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 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)." *Id.* 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. *Id.* 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." *Id.* at [0048].

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." *Id.* 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. *Id.* 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." *Id.* at [0048].

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 to10 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 to10 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).

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).

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).

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).

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).

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.

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 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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EXHIBIT 1008

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

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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 peroxidase 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,

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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 descried 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. CpGenome™ 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 $\geq$3 genes and 7 (35%) patients had methylation in 2 genes. Among the 25 patients with colonic adenoma, only 1 (4%) had methylation in $\geq$ 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

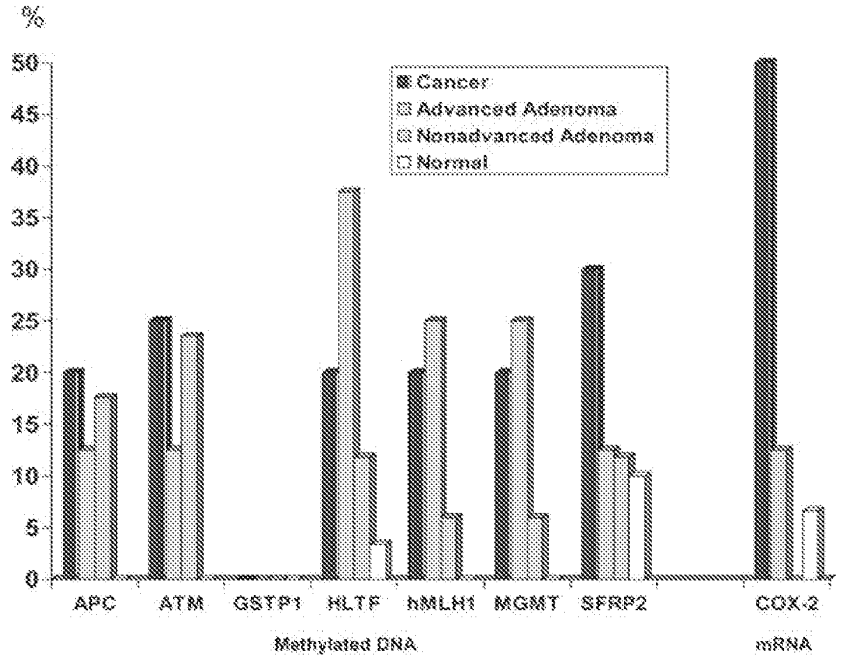


Figure 1. Frequency of detecting methylation changes and COX-2 mRNA in fecal samples.

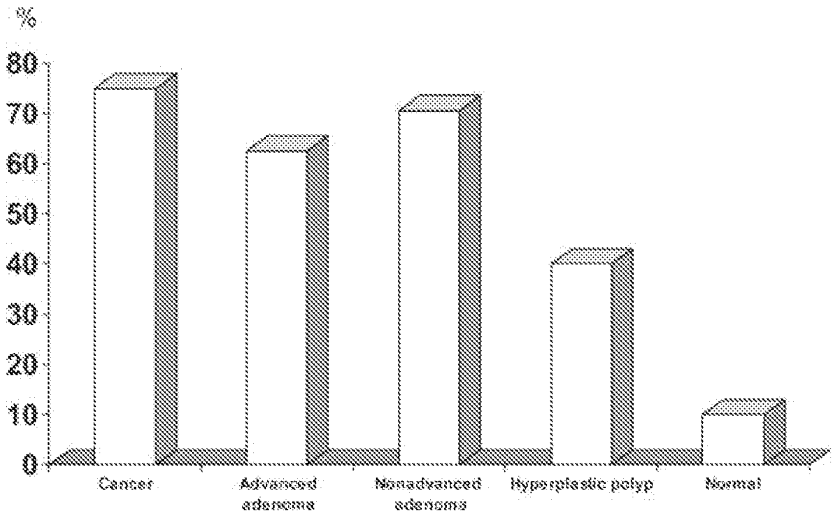


Figure 2. Overall frequency of detecting methylation changes in fecal DNA of patients with different colonic pathology.

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 advanced 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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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/634,607
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				Art Unit	1634
				Examiner Name	WHISENANT
Sheet	1	of	2	Attorney Docket Number	EXCTD-35239.304

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

U.S. PUBLISHED PATENT APPLICATIONS					
Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20070017015	2007-01-25	FINELL	
		20080097238	2008-04-24	LOKTIONOV et al.	

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		EP 1366715	2003-12-03	SENTINEL CH. S.r.L		
		WO 2005/014154	2005-02-17	LOOTS LIMITED		

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				Examiner Name	WHISENANT
Sheet	2	of	2	*Attorney Docket Number*	EXCTD-35239.304

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		Request for Ex Parte Reexamination of U.S. Patent 11,634,781, filed May 22, 2023, 286 pages	
		Declaration of Mr. Anthony P. Shuber, MS, Reexam Application 90/015,237, filed May 22, 2023, 63 pages	

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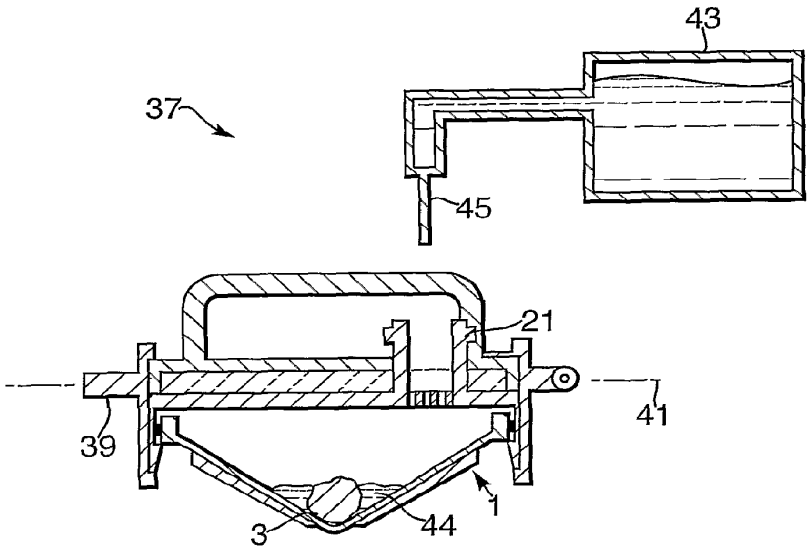
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[Continued on next page]

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



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

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

Field of the Invention

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

Background

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

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

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

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

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

Summary of the Invention

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Brief Description of the Drawings

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

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

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

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

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

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

Detailed Description

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Effect of pre-soaking on SDNAI

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

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

Effect of Rocking Angle

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

Effects of Damage to Stool Surface Layer and of Stool Age

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

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

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

[s] indicates the stool was squashed

[b] indicates the stool had bacterial over-growth

Effect of Wash Solution Volume

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

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

Claims:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Fig.1a.

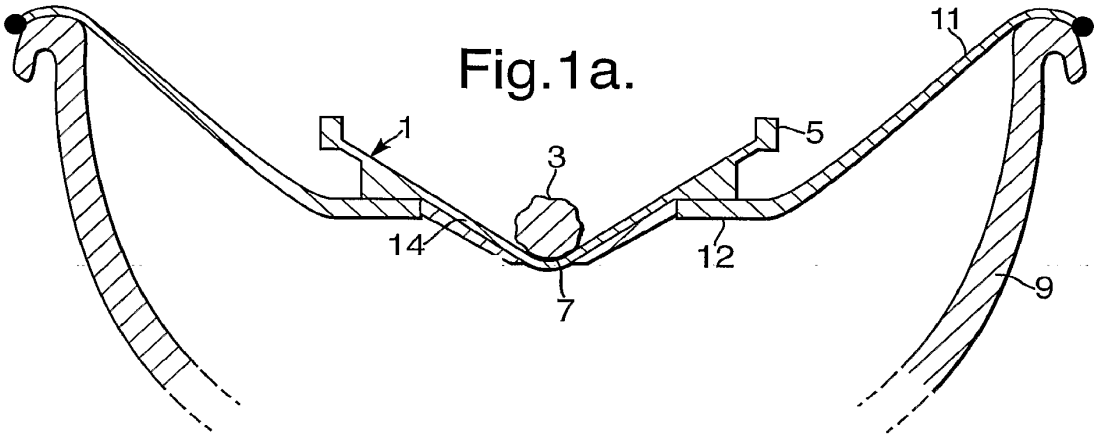


Fig.1b.

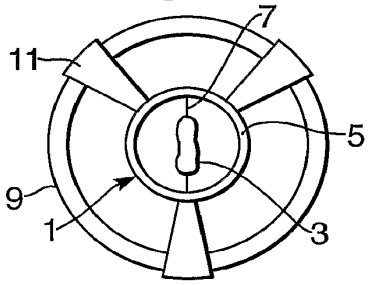


Fig.2.

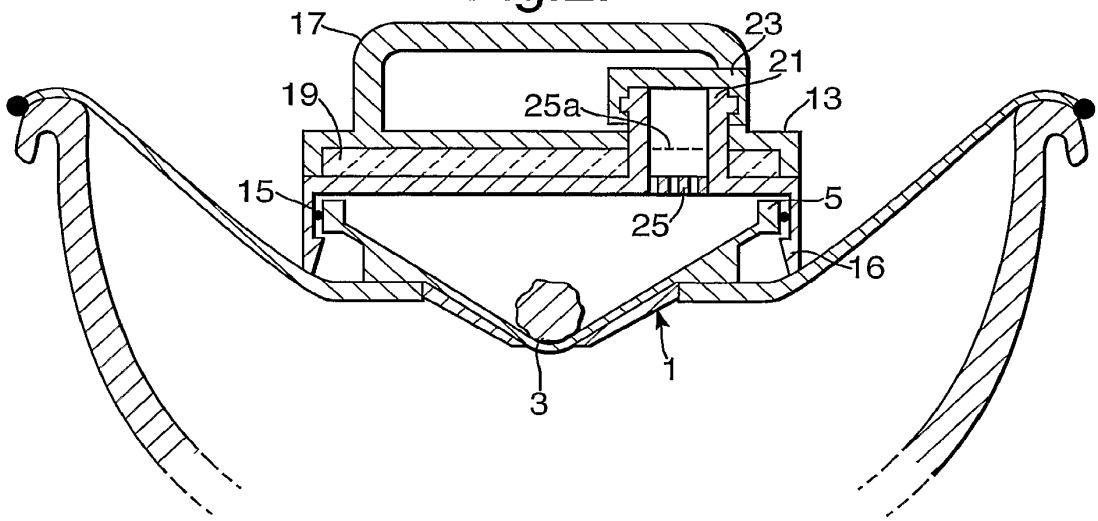


Fig.1c.

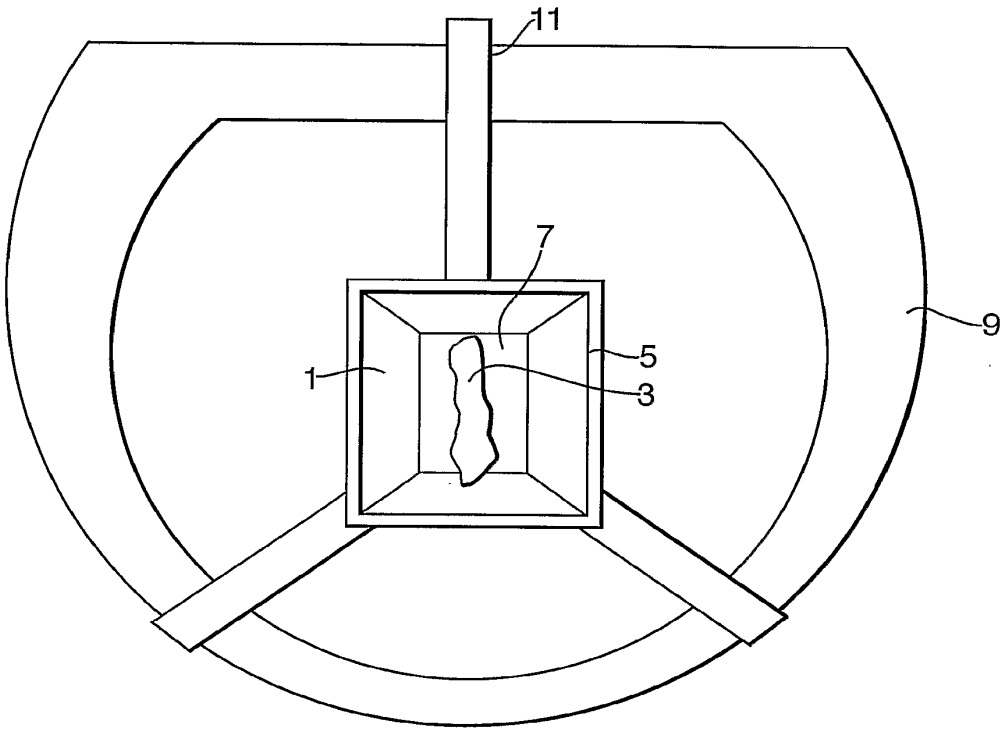


Fig.3a.

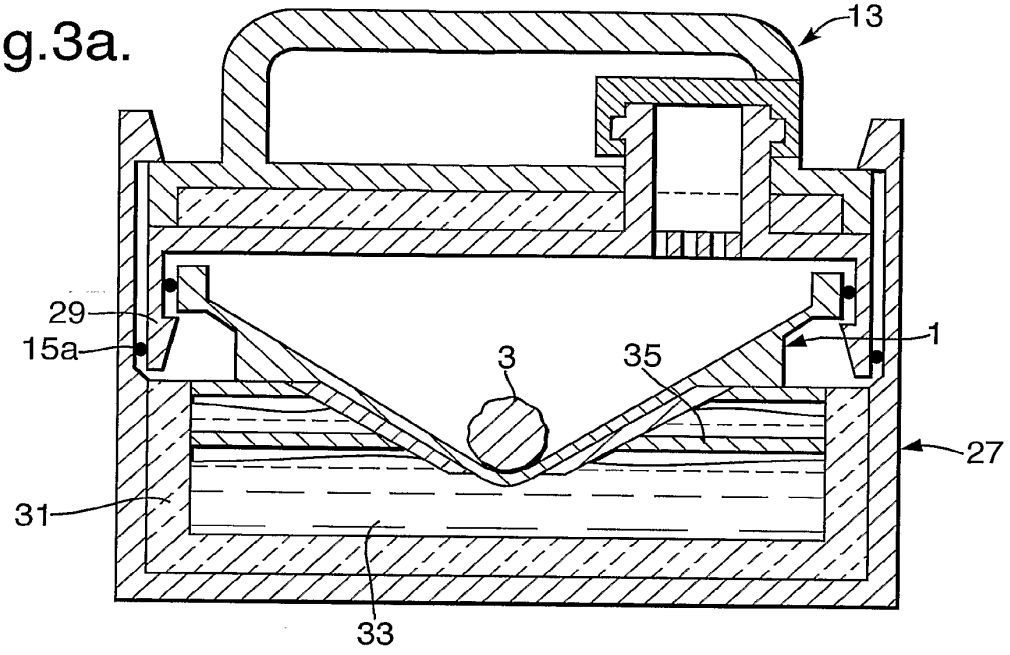

13
29
15a
3
35
1
27
31
33

Fig.3b.

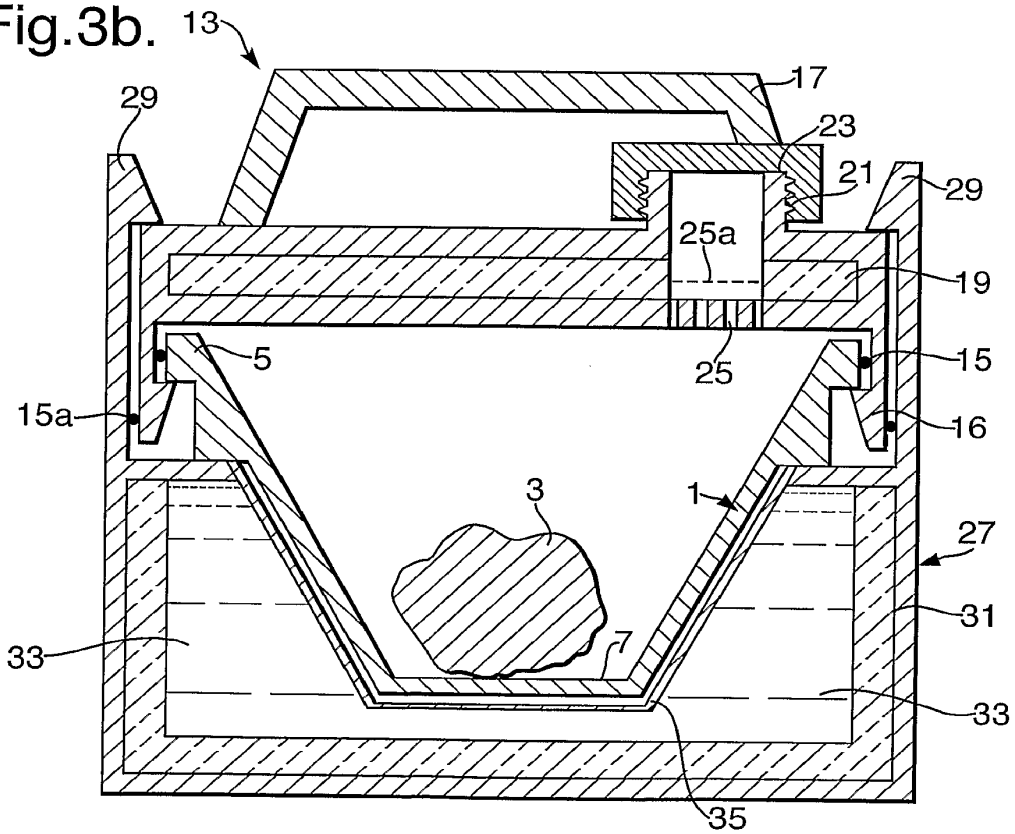

13
17
29
23
21
29
25a
19
5
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15
15a
16
3
1
27
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33
35

Fig.5.

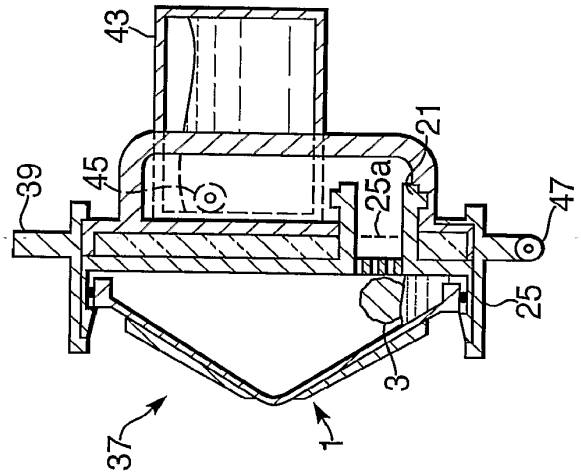
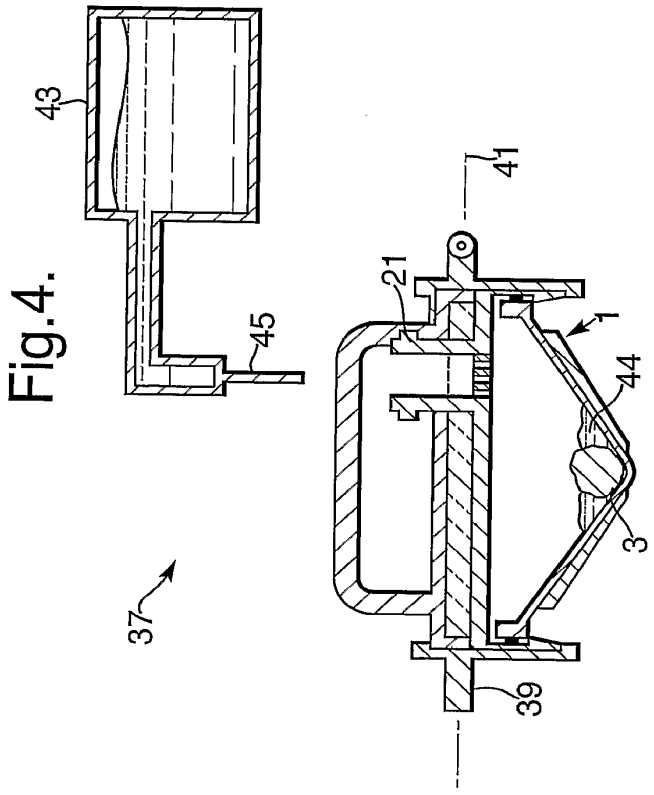


Fig.4.



INTERNATIONAL SEARCH REPORT

International Application No
B2004/003434

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

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
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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
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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

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

Int[illegible]al Application No
PCT/GB2004/003434

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 456 507 A (BRACONNIER THERESE) 12 December 1980 (1980-12-12)	11,14
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ınal Application No
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
15/634,607	**06/28/2023 05:19:15 PM ET**	**EXCTD-35239.304**

Title of Invention

FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1874	FILED BY	Stephanie Filandrinos
PATENT CENTER #	62352099	AUTHORIZED BY	Mary Ann Brow
CUSTOMER #	173254	FILING DATE	06/27/2017
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Joost Louwagie

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 0638	**E20236RH20197129**	**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
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CUSTOMER #	173254	FIRST NAMED INVENTOR	Joost 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_AmJGastro_2007.pdf	8	Non Patent Literature	954 KB
WO2005014154.pdf	31	Foreign Reference	1257 KB
AHLQUIST_AnnInternMed_2008.pdf	23	Non Patent Literature	2103 KB
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Biomarker selection for detection of occult tumour cells in lymph nodes of colorectal cancer patients using real-time quantitative RT–PCR

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Accurate identification of lymph node involvement is critical for successful treatment of patients with colorectal carcinoma (CRC). Real-time quantitative RT–PCR with a specific probe and RNA copy standard for biomarker mRNA has proven very powerful for detection of disseminated tumour cells. Which properties of biomarker mRNAs are important for identification of disseminated CRC cells? Seven biomarker candidates, CEA, CEACAM1-S/L, CEACAM6, CEACAM7-1/2, MUC2, MMP7 and CK20, were compared in a test-set of lymph nodes from 51 CRC patients (Dukes' A–D) and 10 controls. Normal colon epithelial cells, primary tumours, and different immune cells were also analysed. The biomarkers were ranked according to: (1) detection of haematoxylin/eosin positive nodes, (2) detection of Dukes' A and B patients, who developed metastases during a 54 months follow-up period and (3) identification of patients with Dukes' C and D tumours using the highest value of control nodes as cutoff. The following properties appear to be of importance; (a) no expression in immune cells, (b) relatively high and constant expression in tumour tissue irrespective of Dukes' stage and (c) no or weak downregulation in tumours compared to normal tissue. CEA fulfilled these criteria best, followed by CK20 and MUC2.

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Keywords: carcinoembryonic antigen (CEA); CEA cell adhesion molecule (CEACAM); mucin 2 (MUC2); cytokeratin 20 (CK20); matrix metalloproteinase 7 (MMP7).

It is now well established that the earlier the diagnosis of cancer, the better the clinical outcome, through earlier administration of effective and possibly curative treatments (Etzioni *et al*, 2003). For colorectal cancer (CRC) the only curative treatment is surgery. However, even after curative surgery the tumour recurs in many instances. In CRC the prognostic predictor is tumour stage based on histopathologic examination of the resected specimen combined with perioperative findings (Dukes and Bussey, 1958; Lindmark *et al*, 1994; Fleming *et al*, 1997). It has been shown that adjuvant chemotherapy reduces the relative mortality rate by one-third in patients operated for colon cancer in Dukes' Stage C (any TN1-2M0, Stage III) (Moertel *et al*, 1995; Fleming *et al*, 1997). Adjuvant chemotherapy is not routinely given to patients of Dukes' Stage A (T1-2N0M0, Stage I) or Stage B (T3-4N0M0, Stage II) although a substantial number of patients with Dukes' Stage B tumour will die from tumour recurrences. It is considered to be of vital importance to improve the selection criteria in identifying patients who may benefit from adjuvant chemotherapy and intense follow-up protocols.

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A key to these efforts is finding ways to specifically identify disseminated tumour cells in regional lymph nodes. Different techniques, for example analysis of multiple haematoxylin/eosin (H&E)-stained sections, immunohistochemistry, gel-based qualitative reverse transcriptase–polymerase chain reaction (RT–PCR) and more recently real-time quantitative RT–PCR (qRT–PCR), and biomarkers including so-called surrogates of cancer have been utilised (Tsavellas *et al*, 2001; Lotze *et al*, 2005). It can now be concluded that real-time qRT–PCR assay for biomarker mRNA, particularly when used with a specific probe and RNA copy standard, is a superior method for micrometastases detection because it is objective, highly sensitive and quantitative, and has a very wide measuring range (Godfrey *et al*, 2001; Ho *et al*, 2004; Öberg *et al*, 2004). Moreover, this technology lends itself to automation and rapid test performance suitable for a clinical setting (Raja *et al*, 2005).

In a recent study, we used real-time qRT–PCR for detection of disseminated tumour cells in lymph nodes of CRC patients using mRNA for two commonly used tumour markers, carcinoembryonic antigen (CEA) and cytokeratin 20 (CK20) (Öberg *et al*, 2004). The results were promising, particularly for CEA. However, there might be biomarkers even more suitable for this purpose and neither CEA nor CK20 discriminates between dislocated normal intestinal epithelial cells (iECs) and tumour cells with propensity to metastasize. Three kin molecules to CEA are expressed in the

colonic epithelium, that is CEA cell adhesion molecule-1 (CEACAM1), CEACAM6 and CEACAM7 (Horst and Wagener, 2004; Frängsmyr *et al*, 1995, 1999). CEACAM6 has a wider cellular distribution than CEA. However, it has been claimed that tumour levels are higher for CEACAM6 than CEA and that CEACAM6 therefore would be the better tumour marker (Ilantzis *et al*, 2002; Jantscheff *et al*, 2003). CEACAM1 comes with a long (CEACAM1-L) or short (CEACAM1-S) cytoplasmic tail. The long cytoplasmic tail of CEACAM1-L was shown to mediate tumour suppressor function in model systems while the short cytoplasmic tail of CEACAM1-S lacks this property (Hammarström, 1999; Horst and Wagener, 2004). Thus, CEACAM1-S might be a useful biomarker and an increased CEACAM1-S/CEACAM1-L ratio an indicator of tumour transformation. As far as one knows, CEACAM7 shows the same restricted expression pattern as CEA. CEACAM7 mRNA comes in two splice forms (CEACAM7-1 and CEACAM7-2) coding for molecules that differ in the number of extracellular immunoglobulin-like domains. Little is known about their relative expression levels and possible changes in CRC. Mucin 2 (MUC2) is the major mucin in colon (Weiss *et al*, 1996). It is a candidate tumour marker since its expression is restricted to epithelial cells. Finally, matrix metalloproteinase 7 (MMP7)/matrilysin is an interesting candidate biomarker since it is expressed in the invasive front of the primary tumour and MMP7 expression is correlated with capacity to form metastases in CRC transfer experiments (Adachi *et al*, 2001; Yamamoto *et al*, 2003).

The aims of this study were: (1) to determine which properties of a tumour mRNA marker decide if it is suitable for detection of tumour cells in tissues with an excess of immune cells, (2) to find tumour markers that would be complementary to CEA mRNA by improving sensitivity for the identification of disseminated tumour cells in lymph nodes of CRC patients and (3) to find a biomarker for tumour cells with metastasizing capacity.

MATERIALS AND METHODS

Patients

Surgery for CRC was carried out in 51 patients (32 men, 19 women; median age 69 years, range 52–90). Thirty-five tumours were located in colon and 16 in rectum. Seven patients with rectal cancer received preoperative irradiation with 25 Gy. Radical excision of the tumours with wide lymph node dissection was carried out in 42 patients. Nine patients had distant metastases. Five patients received adjuvant chemotherapy. According to Dukes' classification there were six tumours in Stage A (T1-2N0M0, Stage I), 26 in Stage B (T3-4N0M0, Stage II), 10 in Stage C (anyTN1-2M0, Stage III) and nine in Stage D (anyTanyNM1, Stage IV). At follow-up after median 54 months (range: 35–68) 19 patients had died from CRC and eight patients had died from noncancer disease. None of the living patients had tumour recurrence.

Controls included seven men and three women (median age 30 years, range: 18–61) undergoing colorectal surgery for ulcerative colitis (UC; $n = 6$), Crohn's disease (CD; $n = 3$) and rectal prolapse ($n = 1$). Informed consent was obtained from the patients. The local Research Ethics Committee of the Medical Faculty, Umeå University, Sweden, approved our study.

Lymph nodes

One to four lymph nodes were dissected from surgically removed specimens and bisected with separate knives under sterile conditions to prevent RNA cross-contamination. One half of each node was fixed in 10% buffered formalin for routine H&E staining and the other half was snap frozen in liquid nitrogen and stored at −70°C until RNA extraction. In all, 95 lymph nodes were collected from the CRC patients (11 nodes from Dukes' Stage A patients, 55 nodes from Dukes' Stage B patients, 16 nodes from Dukes' Stage C patients and 16 nodes from Dukes' Stage D patients). In the control group 18 nodes were from UC patients, 12 nodes from CD patients and four nodes from the patient with rectal prolapse.

CRC tissue

An approximately $0.5 \times 0.5 \times 0.5$ cm piece was collected from the outer rim of 20 tumour specimens immediately after resection (two Dukes' Stage A, 13 Dukes' Stage B, two Dukes' Stage C and three Dukes' Stage D), snap-frozen and kept at −70°C until RNA extraction.

Epithelial cells from colon tissue

Colonic epithelial cells were isolated from apparently normal tissue constituting the resection margin after surgical removal of tumour in CRC patients and from colon of UC patients subjected to surgical treatment as described earlier (Fahlgren *et al*, 2003b). The isolation procedure yields one fraction enriched in crypt epithelial cells (crypt-iECs) and one fraction enriched in luminal epithelial cells (luminal-iECs).

Cell lines and peripheral blood mononuclear cells

Human cell lines used were: LS174T, T84, HT29, and HCT8 (colon carcinomas), Jurkat and Molt-4 (T-cell lymphomas), CNB6 and KR4 (EBV-transformed B cell lines; a mixture of equal amounts of RNA from the two lines was used in the analyses), U266 (plasmacytoma), U937 (monocyte-like cell line), K562 (erythroblastoid cell line), HL60 (promyelocytic cell line). Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood of healthy adults by Ficoll-Isopaque gradient centrifugation. PBMC were *in vitro* activated by incubation with anti-CD3 mAb OKT3 (50 ng ml^{-1}) in HEPES-buffered RPMI1640 supplemented with 0.4% human serum albumin. PBMC from seven individuals were incubated with the stimulus in parallel cultures for 4, 7, 20, 48 and 72 h, washed, pooled and RNA extracted.

RNA extraction

Total RNA was extracted using the Acid Guanidine Phenol Chloroform (AGPC) method by adding 0.5 ml of a solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl and 0.1 M 2-mercaptoethanol per 25 mg tissue and up to 2.5×10^6 cells in the first homogenization step. Extracted RNA was dissolved in RNAse-free water containing RNAse inhibitor.

Real-time qRT–PCR

Real-time qRT–PCR assays for CEA, CEACAM6, CEACAM1-S, CEACAM1-L, CEACAM7-1, CEACAM7-2, MUC2, MMP7 and CK20 mRNAs were constructed in the laboratory using the TaqMan EZ technology (Applied Biosystems, Foster City, CA, USA). Specific primer pairs were placed in different exons and a dye-labelled probe was placed over the boundary between the two exons in the amplicon. Assays for all markers except MMP7 have been described (Fahlgren *et al*, 2003a; Forsberg *et al*, 2004; Öberg *et al*, 2004). The sequences for the MMP7 primers and probe were: forward primer 5′-GGGAGGCATGAGTGAGCTAC-3′, reverse primer 5′-TCTCCTTGAGTTTGGCTTCTAAA-3′ and probe 5′-TCTTGAGATAGTCCTGAGCCTGTTCCCA-3′. The reporter dye at the 5′-end of each probe was FAM. The quencher dye at the 3′-end was TAMRA for CEA, CEACAM1-L, CEACAM6, CEACAM7-2, CK20 and MMP7, and MGB for CEACAM1-S, CEACAM7-1 and MUC2. Emission from released reporter dye was monitored by the

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ABI Prism 7700 Sequence Detection System (Perkin-Elmer, Wellesley, MA, USA). The RT-PCR profile for all assays except CEACAM7-1 was: 49°C for 2 min, 59°C for 30 min, 94°C for 5 min followed by 45 cycles of 93°C for 20 s and 61°C for 1 min. The RT-PCR profile for CEACAM7-1 was: 49°C for 2 min, 59°C for 30 min, 94°C for 5 min followed by 45 cycles of 93°C for 20 s and 59°C for 1 min. Specific RNA copy standards were prepared as described previously (Fahlgren *et al*, 2003b). Determinations were carried out in triplicates and expressed as copies of mRNA per μl as determined from parallel RT-PCR of serial dilutions of the RNA copy standard. The concentration of 18S rRNA was determined in each sample by real-time qRT-PCR according to the manufacturer's protocol (Applied Biosystems). As no copy standard is available for the 18S rRNA assay the 18S rRNA content was expressed as arbitrary units defined as the amount of 18S rRNA in 1 pg total RNA extracted from PBMC. Results are expressed as mRNA copies per unit of 18S rRNA.

Statistics

The mRNA expression levels in iECs of normal and inflamed colon were compared using two-tailed Mann-Whitney's rank sum test. Comparisons between mRNA expression levels in tumour *vs* normal iECs and between levels of different mRNA species in tumour tissue were performed using Kruskal-Wallis' nonparametric one-way ANOVA with Dunn's multiple comparison *post hoc* test. Analyses of correlations between levels of different mRNA species were performed using two-tailed Spearman rank correlation test. A P-value <0.05 was considered statistically significant.

RESULTS

Biomarker mRNA levels in colonic epithelial cells and CRC tumours

Levels of CEA, CEACAM1-S/L, CEACAM6, CEACAM7-1/2, MUC2, MMP7 and CK20 mRNAs were assessed in RNA extracted from primary tumours representing all four Dukes' stages and from luminal-iECs and crypt-iECs of apparently normal and inflammatory colon using specific real-time qRT-PCR assays with RNA copy standards. The 18S rRNA concentration was determined in each sample and levels are expressed as mRNA copies/18S rRNA unit allowing direct comparison between the different biomarkers. Statistically significant difference in mRNA levels between iECs from normal and inflammatory colon was only seen for MMP7 (see legend to Table 1), therefore the two groups were combined in the comparisons against tumour tissue. Table 1 summarises the results. The mRNA levels of the different biomarkers varied considerably within the tumours, from median 107 mRNA copies/18S rRNA unit for CEA to 0.05 for CEACAM7-1. CEA and CEACAM6 mRNAs were expressed at approximately equal levels in tumours and iECs while the mRNA levels for CEACAM7-1/2, CEACAM1-L, MUC2 and CK20 were significantly decreased in the tumour. Only MMP7 mRNA levels were increased in the tumour compared to iECs (Table 1). Table 2 shows the mRNA levels of the biomarkers in four CRC cell lines. Among the biomarkers CEA exhibited the highest expression level and smallest variation between CRC cell lines.

Biomarker mRNA levels in immune cells

Table 2 summarises the results of analysis of polyclonally activated and resting PBMC and seven immune cell lines. Four biomarker mRNAs were not detected or were detected in trace amounts only in one type of immune cells. These were MUC2, CEA, CEACAM7-2 and CK20. Other biomarkers were expressed at high levels in one or several types of immune cells (CEACAM1-L, CEACAM6, MMP7) or at low levels (CEACAM1-S, CEACAM7-1).

Specificity indexes

To rank the different biomarkers with respect to specificity for CRC we calculated two types of specificity indexes: (I) median value in CRC tumours/highest value in any type of immune cell and (II) median value in CRC tumours/highest value of control lymph nodes (Table 3). As can be seen, the two indexes gave similar values with the exceptions of those for CEACAM6 and CEACAM7-2. CEA was the most specific biomarker followed by CK20. CEACAM6 showed better specificity with index II than index I, while the reverse was true for CEACAM7-2. Indexes for three biomarkers (CEACAM1-L, CEACAM7-1, MMP7) showed no specificity for CRC.

Biomarker mRNA levels in lymph nodes of CRC patients and controls

A total of 129 lymph nodes of 51 CRC patients and 10 controls were analysed for mRNA levels of the biomarkers. Figures 1-3 summarise the results. For each individual only the lymph node

Table 1 Expression levels of mRNAs for CEA, CEACAM1, CEACAM6, CEACAM7, MUC2, MMP7, and CK20 in primary colorectal tumours compared to control colonic epithelial cells

	Tumour		Crypt iEC[a]			Luminal iEC		
mRNA species	Median[b]	IQR[c]	Median	IQR	P-value[d]	Median	IQR	P-value
CEA	107	39-1207	261	225-537	NS	393	212-769	NS
CEACAM6	20	6.5-55	28	14-35	NS	38	13-116	NS
CEACAM1-S	5.1	2.5-14	19	12-34	<0.01	28	12-72	<0.01
CEACAM1-L	1.4	0.43-6.4	16	8.0-40	<0.001	22	10-40	<0.001
CEACAM7-1	0.05	0.005-0.37	0.90	0.67-2.9	<0.001	1.9	1.2-6.0	<0.001
CEACAM7-2	9.0	0.94-32	302	162-468	<0.001	483	361-582	<0.001
MUC2	0.66	0.04-3.4	32	25-56	<0.001	33	17-77	<0.001
MMP7	1.7	0.85-4.3	0.09	0.01-0.25	<0.01	0.09	0.003-16	<0.05
CK20	14	5.5-40	295	178-675	<0.001	162	104-377	<0.001

[a]iEC = intestinal epithelial cells. [b]Median mRNA copies/18S rRNA unit of 20 CRC tumour samples (2 Dukes' Stage A, 13 Dukes' Stage B, 2 Dukes' Stage C and 3 Dukes' Stage D), 14-20 crypt iEC samples and 10-20 luminal iEC samples. Equal numbers of iEC samples were derived from the apparently healthy resection margin of colon from patients operated for CRC and from colon of UC patients. The mRNA expression levels for any of the markers in iEC from normal colon of CRC patients and UC colon, respectively, did not differ significantly from each other with the exception of MMP7 mRNA. MMP7 mRNA levels were significantly higher ($P<0.01$) in the iECs from UC patients compared to iEC from the resection margin of CRC patients. MMP7 values for iEC in the Table include data from 10 CRC and 10 UC patients. [c]IQR = interquartile range from the 25 to the 75 percentile. [d]P-value obtained by comparing mRNA expression levels in tumours with those in crypt iECs, and luminal iECs using Kruskal-Wallis nonparametric one-way ANOVA with Dunn's multiple comparison *post hoc* test. NS = not significant, that is P-value >0.05.

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Table 2 Expression levels of mRNA for CEA, CEACAM1, CEACAM6, CEACAM7, MUC2, MMP7, and CK20 in CRC cell lines and different types of immune cells

		Biomarker mRNA								
Cells	**Origin**	**CEA**	**CEACAM6**	**CEACAM1-S**	**CEACAM1-L**	**CEACAM7-1**	**CEACAM7-2**	**MUC2**	**MMP7**	**CK20**
HT29	Colon iEC	32[a]	43	22	17	0.003	0.3	0.01	53	85
LS174T	Colon iEC	328	81	1.2	1.4	0.5	38	4.3	3.4	0.02
T84	Colon iEC	33	1.0	0.9	0[b]	0.003	0.07	0.5	2.3	33
HCT8	Colon iEC	32	0.5	0.003	0	0	0.1	0.02	0.2	0.05
PBMC		0	0.05	0	0.3	0.01	0	0	0.001	0
Act. PBMC		0	0	0.001	0.8	0	0	0	2.0	0
Jurkat	T cell	0	0	0.004	0.1	0	0	0	0.004	0
Molt-4	T cell	0	0	0.002	0.05	0	0	0	0	0
CNB6+KR4	B cell	0	0	0.008	1.1	0.05	0	0	0.6	0
U266	Plasma cell	0	0	0.001	0.02	0	0	0	0	0
U937	Monocyte	0.005	0.3	0.002	0.03	0	0	0	0.9	0.003
HL60	Granulocyte	0	1.8	0	ND	0	0.001	ND	0	0
K562	Pre-erythrocyte	0	0	0.006	0.08	0	0	0	0	ND

[a]Expression level of the indicated mRNA species expressed as mRNA copies/18S rRNA unit. [b]Values below 0.001 mRNA copies/18S rRNA unit are recorded as 0. ND = not determined.

Table 3 Specificity indexes for tumour marker mRNAs

mRNA species	**Median CRC tumour value/highest value of any immune cell type**	**Median CRC tumour value/highest value of control lymph nodes**
CEA	21 400	53 500
CEACAM6	11	2000
CEACAM1-S	638	39
CEACAM1-L	1.3	0.4
CEACAM7-1	1.0	6.3
CEACAM7-2	9000	90
MUC2	>660	942
MMP7	0.9	1.1
CK20	4667	15 555

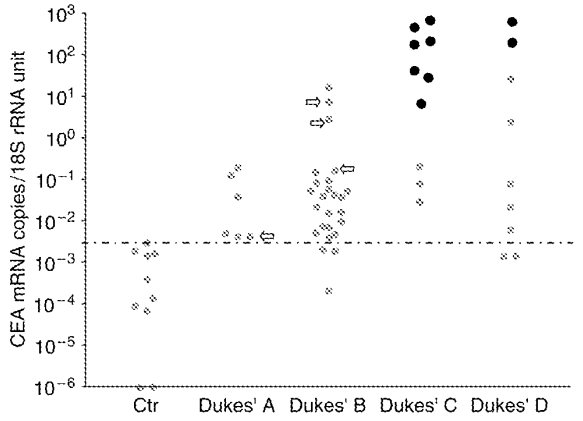


Figure 1 CEA mRNA levels in lymph nodes as determined by real-time qRT–PCR for CEA mRNA and 18S rRNA content in the samples. Each patient is represented by the lymph node with the highest CEA mRNA level (dots). Arrows indicate the four patients with Dukes' Stage A and B tumours, who had died from CRC during the 54 months (range, 35–68 months) follow-up time. Large black dots indicate lymph nodes that had tumour cells identified by H&E staining and small grey dots indicate H&E negative nodes. Results updated from Öberg *et al*, 2004.

with the highest level of the indicated mRNA species is shown. For each biomarker, a cutoff level is set at the highest control node.

CEA mRNA showed excellent discrimination between nodes from CRC patients and controls (Figure 1). Practically all CRC nodes were above the cutoff level independent of Dukes' Stage and all H&E positive lymph nodes had very high CEA mRNA levels. Moreover, the three Dukes' B patients that have died from their CRC within the 54 months follow-up period displayed highly elevated CEA mRNA levels (arrows in figure). The single Dukes' A patient that has died from her CRC showed a borderline value (arrow).

CEACAM1-S mRNA (Figure 2A) showed some discrimination between CRC and controls. Nodes of 1/6 Dukes' A, 5/24 Dukes' B, 7/10 Dukes' C and 2/8 Dukes' D patients were above cutoff and all but one H&E positive nodes were identified as positive by CEACAM1-S mRNA level. Moreover, two of the three Dukes' B patients, but not the Dukes' A patient, that have died from their CRC within the follow-up period were identified. Note also that the total range of values is much smaller for CEACAM1-S than for CEA, that is four and seven orders of magnitude, respectively, and that the CEACAM1-S levels in controls are almost 100 times higher.

No discrimination between CRC and control nodes was seen with CEACAM1-L mRNA (Figure 2B). The CEACAM1-L mRNA levels of control nodes were very high, which is consistent with the high levels of CEACAM1-L mRNA in immune cells (Table 2).

CEACAM7-1 mRNA was not a useful marker (Figure 2C) while CEACAM7-2 mRNA, which was expressed at about 100 times higher concentration, displayed some discriminating power between CRC patients and controls (Figure 2D). However, several H&E positive nodes were missed and one of the three Dukes' B patients and the Dukes' A patient that have died from CRC within the follow-up period were missed.

MUC2 mRNA showed good discrimination between CRC nodes and control nodes comparable to that of CEA mRNA. All but one of the H&E positive nodes and all three Dukes' B patients who had died from CRC had values above the cutoff (Figure 3A). Interestingly, MUC2 mRNA values are very low in controls and the range of values in CRC is quite large.

Similarly, CK20 mRNA showed good discrimination between nodes from CRC patients and controls (Figure 3B). All H&E positive nodes as well as the three Dukes' B patients who have died

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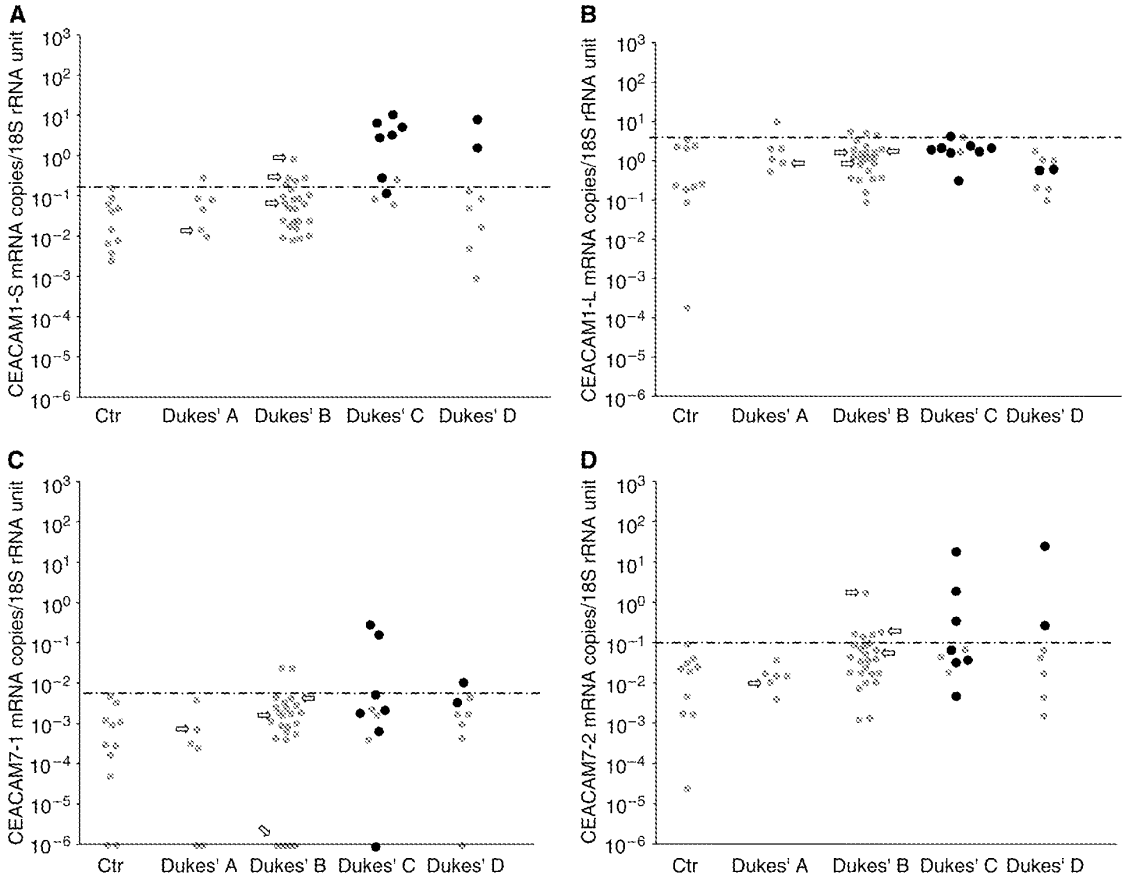


Figure 2 CEACAM1-S (**A**), CEACAM1-L (**B**), CEACAM7-1 (**C**), and CEACAM7-2 (**D**) mRNA levels in lymph nodes of CRC and control patients. Each patient is represented by the lymph node with the highest level of the respective mRNA species. For explanation of dots and arrows see legend to Figure 1.

from CRC during the follow-up period were identified as positive. CK20 mRNA levels in CRC nodes varied over a wide range and control nodes were low.

CEACAM6 mRNA displayed fairly good ability to discriminate between CRC patients and controls (Figure 3C). However, only two of the three Dukes' B patients that have died from their CRC within the follow-up period had elevated CEACAM6 levels. All H&E positive nodes displayed CEACAM6 mRNA levels above cutoff.

MMP7 mRNA displayed poor discriminating power between CRC patients and controls (Figure 3D).

Correlation between different biomarker mRNA levels in lymph nodes of CRC patients and controls

The four biomarker mRNAs which showed the best discriminating power between CRC and control nodes and which were not appreciably expressed in immune cells namely CEA, CEACAM7-2, CK20 and MUC2 were analysed by pair-wise comparisons of biomarker levels in all 129 lymph nodes. Each Dukes' Stage and controls were also analysed separately. Table 4 shows correlation coefficients (r-values) and significance levels (P-values) for all six comparisons. Biomarker mRNA values were significantly correlated if all nodes were compared with r-values ranging from 0.47 to 0.76. When the different stages of CRC were analysed separately it was noted that lymph nodes from Dukes' C and Dukes' D patients showed r-values $\geqslant 0.5$ and P-values $\leqslant 0.05$ for all marker combinations except one indicating that it was the same cells that expressed the four markers. The comparisons furthermore demonstrate that CEACAM7-2 is also expressed in a separate cell population normally present in lymph nodes. The results also indicate that control nodes harbour low numbers of two cell populations of epithelial origin, one expressing CEA, CK20 and CEACAM7-2 and another expressing MUC2, CK20 and CEACAM7-2.

DISCUSSION

In this study, we have explored the possibility of using real-time qRT-PCR as an alternative, or adjunct, to the classical H&E staining method for detection of disseminated tumour cells in regional lymph nodes. There are several reasons to search for alternatives. Firstly an inherent problem with histochemical as well as immunohistochemical methods is that of sampling. Usually, only one or a few 6 μm thick sections are analysed covering $<1\%$ of the lymph node volume. To analyse the number of sections that would be needed to properly cover the node becomes impossible in practice. In the qRT-PCR study reported here, we extracted RNA from one half of the lymph node. A second problem specific for H&E is to decide on the basis of morphology alone whether a small number of cells indeed are tumour cells. Thirdly, and in contrast to H&E and immunohistochemistry, the qRT-PCR assay is very sensitive and objective and has a very large measuring range. With the appropriate choice of primers and probes, it can be made highly specific for the chosen marker mRNA. Moreover, since

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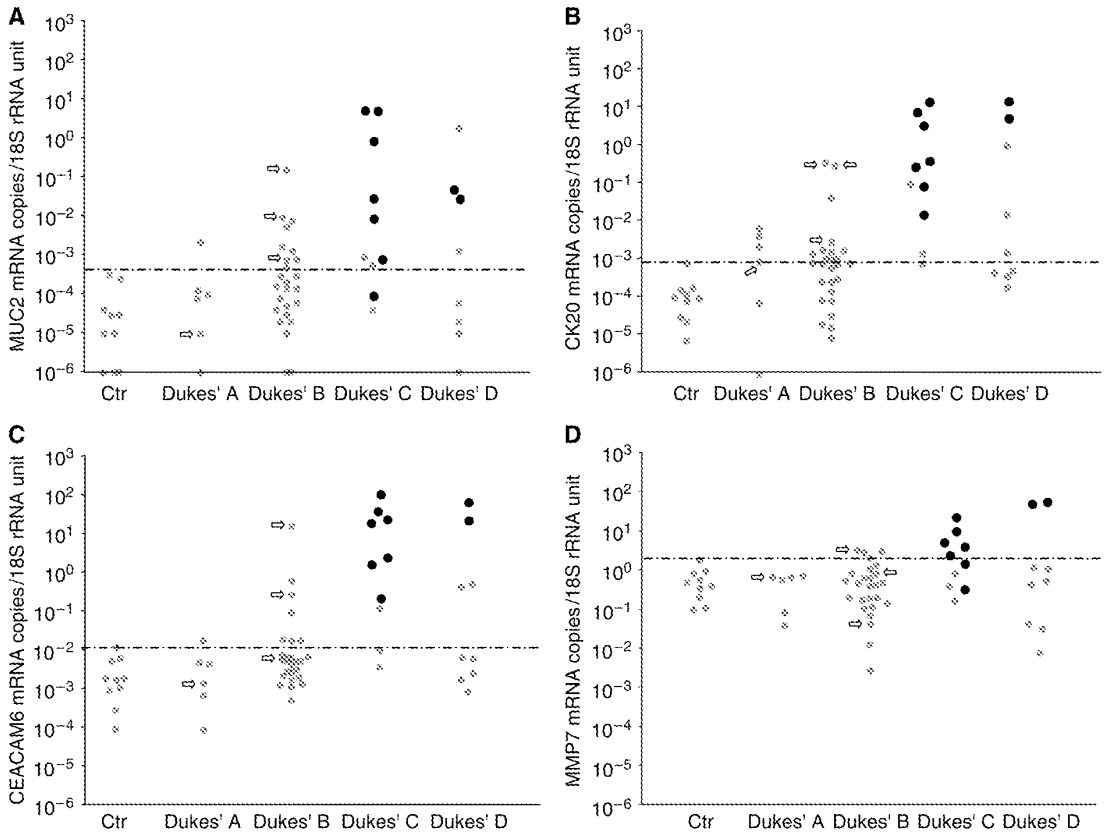


Figure 3 MUC2 (**A**), CK20 (**B**), CEACAM6 (**C**), and MMP7 (**D**) mRNA levels in lymph nodes of CRC and control patients. Each patient is represented by the lymph node with the highest level of the respective mRNA species. For explanation of dots and arrows see legend to Figure 1.

Table 4 Correlation between expression levels of biomarker CEA, CEACAM7-2, CK20 and MUC2 mRNAs in lymph nodes of CRC patients and controls

	All lymph nodes		Controls		Dukes' A		Dukes' B		Dukes' C		Dukes' D	
Compared mRNA species	***r*[a]**	***P*-value[a]**	***r***	***P*-value**	***r***	***P*-value**	***r***	***P*-value**	***r***	***P*-value**	***r***	***P*-value**
CEA *vs* CK20	0.76	<0.0001	0.47	0.005	0.80	0.005	0.58	<0.0001	0.70	0.004	0.76	0.0006
MUC2 *vs* CK20	0.62	<0.0001	0.50	0.004	0.62	0.04	0.45	0.001	0.69	0.007	0.60	0.05
CEA *vs* MUC2	0.71	<0.0001	0.33	NS	0.60	NS	0.55	<0.0001	0.78	0.0006	0.80	0.003
CEA *vs* CEACAM7-2	0.62	<0.0001	0.64	<0.0001	0.42	NS	0.40	0.004	0.54	0.03	0.89	0.0003
CEACAM7-2 *vs* CK20	0.47	<0.0001	0.53	0.001	0.37	NS	0.16	NS	0.52	0.05	0.85	0.002
CEACAM7-2 *vs* MUC2	0.55	<0.0001	0.43	0.02	0.44	NS	0.43	0.003	0.41	NS	0.80	0.005

[a]*r*- and *P*-values obtained by pair-wise comparing the expression levels of the indicated mRNA species in lymph nodes of CRC patients and controls using two-tailed Spearman rank correlation test. Numbers of lymph nodes in the analysis were 120–129 for all lymph nodes, 33–34 for controls, 10–11 for Dukes' A patients, 48–52 for Dukes' B patients, 15–16 for Dukes' C patients and 12–16 for Dukes' D patients. NS = not significant, *P*-value > 0.05.

analyses are performed at the mRNA level instead of at the protein level positive results are likely to reflect the presence of living tumour cells. It has been shown that dendritic cells/macrophages can transport protein components from apoptotic epithelial cells to lymph nodes (Bonnotte *et al*, 2000; Huang *et al*, 2000). A yet unresolved question in using qRT–PCR for the purpose of detecting disseminated tumour cells in regional lymph nodes is, however, which biomarker mRNA to use.

A major goal of this study was to determine which properties of a tumour marker mRNA are of importance for the detection of disseminated colon tumour cells in the presence of an excess of immune cells for example in regional lymph nodes and blood. We, therefore, investigated a number of biomarkers, that are expressed in colon adenocarcinoma cells and compared their expression levels in a test-set of lymph nodes from CRC patients of different Dukes' stages and controls as well as in primary tumours, in epithelial cells of normal and inflamed colon and in different types of immune cells.

We found that CEACAM1-S/L, CEACAM7-1/2, MUC2 and CK20 mRNAs were expressed at lower levels in the primary tumour compared to normal colon. CEACAM1 and CEACAM7 mRNAs have previously been shown to be downregulated in CRC using semiquantitative methods (Neumaier *et al*, 1993; Nollau *et al*, 1997). In contrast, neither CEA nor CEACAM6 mRNA levels were

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decreased in tumour tissue. MMP7 was the only marker that exhibited increased levels in tumours. However, the levels were still low compared to CEA. CEA and CEACAM1-S levels displayed the smallest variation between individual primary tumour tissue samples and there was no tendency for changes in relation to Dukes' stages for these two markers. Of all biomarkers CEA mRNA was expressed at the highest levels in tumours followed by CEACAM6. The high levels of these two markers could possibly be explained by the fact that CEA and CEACAM6 are expressed both in columnar epithelial cells and goblet cells in normal colon while CEACAM1 and CEACAM7 are expressed in columnar epithelial cells only and MUC2 in goblet cells only (Weiss *et al*, 1996; Frängsmyr *et al*, 1999).

It can be argued that no or only marginal expression of the biomarker in immune cells should be of utmost importance for successful detection of tumour cells in lymph nodes. Indeed, three of the four biomarkers that were expressed in only trace amounts in immune cells, that is CEA, CK20 and MUC2, showed good discriminatory capacity between nodes from CRC patients and controls. Owing to its low expression in immune cells one would have predicted excellent discriminatory capacity also by CEACAM7-2 but this was not the case. Possibly CEACAM7-2 is expressed in some additional cell type in lymph nodes, for example endothelial cells or stromal cells.

CEA mRNA showed excellent separation between CRC patients and controls. MUC2 and CK20 mRNAs had almost the same discriminating power as CEA mRNA. The Dukes' B patients who developed metastatic disease during the follow-up period were all detected by the three markers but only for CEA were all Dukes' C nodes above the cutoff value. Moreover, one H&E positive lymph node was missed by the MUC2 mRNA assay.

It was somewhat unexpected that MUC2 gave essentially the same result as CEA although in normal colon MUC2 is confined to goblet cells and not expressed in columnar epithelial cells. Does all CRC tumour disseminated to the lymph node contain goblet cell-like elements? We do not know whether this is the case. Possibly MUC2 expression can be induced by external agents in CRC tumour cells. It was recently shown that bile acids induce MUC2 overexpression in human colon carcinoma cells (Song *et al*, 2005). Furthermore, we found that the inflammatory state of the small intestinal mucosa in patients with active celiac disease is associated with ectopic production of MUC2 by enterocytes (Forsberg *et al*, 2004). Thus, the cytokine milieu in the nodes might upregulate MUC2 expression in the iECs.

Although CEA and CEACAM6 behaved very similar CEA had a higher discriminatory power. This was due to CEA that, contrary to expectation, had higher expression level in tumour cells than CEACAM6 and that CEACAM6 was expressed in myeloid immune cells with the risk that tumour cells expressing fairly low levels of this marker would drown in the immune cell background of the node.

The two markers for tumour cell 'aggressiveness' studied here, that is MMP7 and CEACAM1-S, were not suitable for analysis of lymph nodes. MMP7 because its expression was induced in activated T lymphocytes, a cell type which is likely to be prominent in lymph nodes. CEACAM1-S was not suitable because (1) it was expressed in several types of immune cells although at low levels and (2) the very high expression level of CEACAM1-L in immune cells precludes meaningful calculation of CEACAM1-S: CEACAM1-L ratios in lymph nodes.

In conclusion, the finding that three independent biomarker mRNAs, CEA, MUC2 and CK20, gave almost the same results with highly selective expression in cells of epithelial origin strongly support the notion that disseminated tumour cells can be successfully detected in regional lymph nodes by this technique. CEA mRNA appears to be the best choice as single marker due to its remarkably high expression level in colorectal tumour cells.

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Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia

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Abstract

Background—Stool DNA testing is a new approach to colorectal cancer detection. Few data are available from the screening setting.

Objective—To compare stool DNA and fecal blood testing for detection of screen-relevant neoplasia (curable-stage cancer, high-grade dysplasia, or adenomas >1 cm).

Design—Blinded, multicenter, cross-sectional study.

Setting—Communities surrounding 22 participating academic and regional health care systems in the United States.

Participants—4482 average-risk adults.

Measurements—Fecal blood and DNA markers. Participants collected 3 stools, smeared fecal blood test cards and used same-day shipment to a central facility. Fecal blood cards (Hemoccult and HemoccultSensa, Beckman Coulter, Fullerton, California) were tested on 3 stools and DNA assays on 1 stool per patient. Stool DNA test 1 (SDT-1) was a precommercial 23-marker assay, and a novel test (SDT-2) targeted 3 broadly informative markers. The criterion standard was colonoscopy.

Results—Sensitivity for screen-relevant neoplasms was 20% by SDT-1, 11% by Hemoccult ($P = 0.020$), 21% by HemoccultSensa ($P = 0.80$); sensitivity for cancer plus high-grade dysplasia did not differ among tests. Specificity was 96% by SDT-1, compared with 98% by Hemoccult ($P < 0.001$) and 97% by HemoccultSensa ($P = 0.20$). Stool DNA test 2 detected 46% of screen-relevant neoplasms, compared with 16% by Hemoccult ($P < 0.001$) and 24% by HemoccultSensa ($P < 0.001$). Stool DNA test 2 detected 46% of adenomas 1 cm or larger, compared with 10% by Hemoccult ($P < 0.001$) and 17% by HemoccultSensa ($P < 0.001$). Among colonoscopically normal patients, the positivity rate was 16% with SDT-2, compared with 4% with Hemoccult ($P = 0.010$) and 5% with HemoccultSensa ($P = 0.030$).

Limitations—Stool DNA test 2 was not performed on all subsets of patients without screen-relevant neoplasms. Stools were collected without preservative, which reduced detection of some DNA markers.

Conclusion—Stool DNA test 1 provides no improvement over HemoccultSensa for detection of screen-relevant neoplasms. Stool DNA test 2 detects significantly more neoplasms than does Hemoccult or HemoccultSensa, but with more positive results in colonoscopically normal patients. Higher sensitivity of SDT-2 was particularly apparent for adenomas.

Colorectal cancer remains the second most common cause of death among the types of cancer (1). Although screening reduces colorectal cancer mortality (2–6), observed reductions have been modest (6, 7) and more than one half of adults in the United States have not received screening (8). More accurate, user-friendly, and widely distributable tools have the potential to improve screening effectiveness, acceptability, and access.

Several molecular approaches to screening stool for colorectal cancer have been studied and reviewed (9, 10), and stool DNA testing has been jointly endorsed by the American Cancer Society, the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College

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of Radiology (11). The advantages of stool DNA testing include noninvasiveness, absence of bowel preparation or dietary restrictions, and ease of access via mail courier. However, the reported accuracy of stool DNA tests for the detection of colorectal neoplasia varies. In clinical studies that used different assays and selected groups (12–20), sensitivities ranged from 62% to 100% for colorectal cancer and 27% to 82% for advanced adenomas, with specificities ranging from 82% to 100%. In the only reported multi-center study on asymptomatic average-risk patients (21), a precommercial multitarget DNA assay (SDT-1, a prototype of PreGenPlus, EXACT Sciences, Marlborough, Massachusetts) detected 52% of cases of colorectal cancer, compared with 13% by Hemoccult ($P = 0.003$), at specificities of 94.4% and 95.2%, respectively.

The accuracy of stool DNA testing is influenced by both biological and technical factors. A panel of markers must be used to accommodate the molecular heterogeneity of colorectal neoplasia, and marker selection critically affects discrimination (9). Unlike occult bleeding, which is intermittent (22), DNA markers seem to be shed continuously by exfoliation (23). Thus, the multiple stool sampling practiced with fecal occult blood tests may not be necessary with stool DNA tests. However, recovery of the minute quantities of human DNA and assay of tumor-specific DNA alterations from stool present technical challenges and require exquisite laboratory sensitivity to achieve optimal detection rates.

Our primary aim was to compare the precommercial stool DNA test (SDT-1), which was studied by Imperiale and colleagues (21), with widely used fecal occult blood tests for the detection of screen-relevant neoplasia, defined as curable-stage colorectal cancer (no distant metastases), high-grade dysplasia, or adenomas larger than 1 cm. A secondary aim was to explore neoplasm detection by another stool DNA test 2 (SDT-2), which uses a more broadly informative marker panel.

Methods

Table 1 lists the genes used in our test panels and defines several key terms.

Design

We conducted this multicenter, prospective, triple-blinded trial, targeting average-risk persons, from 2001 to 2007. A group of national experts on colorectal cancer screening advised on study design, and institutional review boards at each site approved the study. Because we did not know the effect of diet and medications on DNA assays, patients were randomly assigned at entry to group A (restriction of red meat and therapeutic doses of nonsteroidal anti-inflammatory drugs for 3 days before and during stool collections) or group B (no such restrictions). All patients were asked not to ingest vitamin C for the 3 days before and during stool collections. For the companion test, we chose Hemoccult (Beckman Coulter, Fullerton, California), the most widely used fecal occult blood test, which was used in the trials that established the benefit of screening for fecal occult blood (2–4). As a second companion test, we chose the next-generation guaiac test HemoccultSensa (Beckman Coulter). 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. All

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patients who completed stool collections also had colonoscopy, which served as the criterion standard. We did not have access to data until after they had been analyzed by statisticians and released by a data monitoring board.

Participants

We recruited asymptomatic persons age 50 to 80 years who were at average risk for colorectal cancer from communities surrounding 22 participating academic and regional health care systems through direct mail and multi-media advertisements. The exclusion criteria were structural colorectal evaluation (endoscopic or radiographic) within 10 years; fecal blood testing within 1 year; overt rectal bleeding within 1 month; previous colorectal resection; aerodigestive cancer within 5 years; inability to stop therapeutic doses of nonsteroidal anti-inflammatory drugs or anticoagulants; coagulopathy; contraindications to colonoscopy; chemotherapy within 3 months; high-risk conditions for colorectal cancer, such as familial adenomatous polyposis, the Lynch syndrome, or other cancer syndromes; previous colorectal cancer or adenoma; inflammatory bowel disease; or more than 2 first-degree relatives with colorectal neoplasia. Study assistants at each site registered participants and randomly assigned them by using a Web-based management system; distributed fecal blood test cards, stool collection containers, and colonoscopy preparation materials; and provided instructions.

Stool Collection and Processing

Patients 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. We froze the first stool from each participant whole at −80 °C on receipt and sent it in batches on dry ice to EXACT Sciences (Marlborough, Massachusetts) for DNA assay; each of the subsequent 2 stools were archived in aliquots at −80 °C. If the first stool weighed less than 30 g or was received more than 48 hours after defecation, it was rejected for DNA analysis and the second or third stool (if it met inclusion criteria) was sent for DNA assay.

Stool Assays

DNA Testing—All 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 *K-ras* 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 *K-ras* (12), *APC* scanning (25), and vimentin gene methylation (20) assays. We defined any

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positive component marker result according to the manufacturer's preestablished criteria as a positive test result.

Occult Blood Testing—The 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.

Colonoscopy

After cathartic preparation, experienced endoscopists performed colonoscopy in all patients. If the examination did not reach the cecum or inspected less than 90% of the mucosa, the patient was disqualified. Endophotographs documented cecal intubation, and the size and location of all lesions were recorded. Costs not covered by third parties were reimbursed by study funding.

Pathologic Examination

Local pathologists examined all endoscopically or surgically sampled lesions. A gastrointestinal pathologist at the coordinating site reexamined all lesions to confirm diagnosis. Classification discrepancies of screen-relevant neoplasms were adjudicated by a second expert pathologist. We categorized patients with multiple neoplasms according to the most advanced lesion. For assay of markers in screen-relevant neoplasms, DNA was extracted from microdissected tissue.

Statistical Analysis

We calculated sample size to ensure adequate power to detect differences in sensitivity comparisons. We powered the study to ensure an adequate number of cases of curable-stage colorectal cancer and high-grade dysplasia and assumed their combined prevalence to be at least 1.5%. A sample size of 2900 would yield an expected 43 curable-stage cancer or high-grade dysplasia cases, which would provide 90% power to detect a 35% improvement in sensitivity of SDT-1 over the Hemoccult test by using a 2-sided McNemar test with $\alpha = 0.05$ (assuming Hemoccult sensitivity of 25%). The protocol specified interim analyses at one half and three quarters of full enrollment to see whether it was necessary to stop the study early if test sensitivities differed significantly or to revise sample size requirements on the basis of observed prevalence of the target lesion. At the first interim analysis, lesion prevalence was lower than expected, and we readjusted the sample size to 4434 patients. However, before we completed enrollment, the manufacturer altered the SDT-1 assay, which prompted an unplanned interim analysis after 2497 patients. On the basis of these interim results, we stopped SDT-1 testing and began doing the SDT-2 test.

To accomplish a secondary aim of this trial (to see whether restricting diet and medication affects the specificity of the SDT test), we randomly assigned persons to pretest restrictions or no restrictions. The sample size calculated for the sensitivity comparison provided 85%

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power to detect a 4% difference in specificity between randomization groups. Because SDT specificity was the same in both groups, we pooled the results for all analyses.

We included all patients tested with SDT-1. We compared stool test sensitivities and specificities by using the McNemar test. We used a chi-square test or the Fisher exact test to compare baseline characteristics between cohorts and assay performance in subsets of patients. All *P* values are 2-sided.

Per agreement with EXACT Sciences, we did the SDT-2 test on all patients with cancer, high-grade dysplasia, and adenomas larger than 2 cm from the full enrollment period as well as on a random sample of 50 patients with 1- to 2-cm adenomas and 75 with normal colonoscopy results. To estimate the population-level sensitivity for the SDT-2 test, we used all case patients tested with SDT-2 and reweighted the calculation to be proportional to the observed prevalence of each screen-relevant neoplasia category in the entire population with screen-relevant neoplasias. Because we did not do the SDT-2 test on all subsets without screen-relevant neoplasia, we could not calculate specificity for screen-relevant neoplasia. To compare test positivity rates in patient subsets, we used the McNemar test.

Role of the Funding Source

The National Cancer Institute funded this study and monitored conduct. EXACT Sciences performed DNA assays at no cost, and Beckman Coulter provided Hemoccult and HemoccultSensa cards at no cost. EXACT Sciences limited SDT-2 coverage to screen-relevant neoplasms and a subset of normal control participants. Neither company influenced study oversight, data analysis, or reporting.

Results

Patients

Of the 4482 persons enrolled, 3764 (84%) were evaluable. We excluded 545 patients because of cancellations, protocol violations, or ineligibility; 171 because of incomplete colonoscopies; and 2 because of distant metastases (Figure 1). Table 2 shows demographic and colorectal lesion characteristics. We found screen-relevant neoplasms in 290 (7.7%) patients; 39 had nonmetastatic cancer or high-grade dysplasia and 251 had adenomas that were 1 cm or larger. Major complications from colonoscopy occurred in 4 patients; no procedure-related deaths were reported.

Occult Blood Testing: Hemoccult versus HemoccultSensa

Detection 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 screen-relevant 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

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($P = 0.100$). 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%) ($P = 0.001$).

Stool DNA versus Occult Blood Testing

SDT-1 versus Occult Blood Testing—Based on the first 2497 evaluable participants (Table 3), the sensitivity of SDT-1 for screen-relevant neoplasia was higher than that of Hemoccult (20% [CI, 14% to 26%] vs. 11% [CI, 6% to 16%]; $P = 0.020$) but not that of HemoccultSensa (21% [CI, 15% to 27%]; $P = 0.80$). For all target lesion groupings, specificities were slightly but significantly lower for SDT-1 than for Hemoccult but not HemoccultSensa, and the positive likelihood ratios for SDT-1 were lower than for either Hemoccult or HemoccultSensa for the more advanced groupings of screen-relevant neoplasms (Table 3).

Based on test positivity data in subsets of screen-relevant neoplasms (Table 4), SDT-1 had higher detection rates than Hemoccult for 1- to 2-cm adenomas but not for any other subset. Stool DNA test 1 had a lower positivity rate for detecting invasive cancer than did HemoccultSensa (25% [CI, 5% to 57%] vs. 75% [51% to 100%]; $P = 0.010$).

SDT-2 versus Occult Blood Testing—Table 3 shows the sensitivity of SDT-2, Hemoccult, and HemoccultSensa for screen-related neoplasia. The weighted estimate of SDT-2 sensitivity for screen-relevant neoplasms was 40% (CI, 32% to 49%), compared with the observed Hemoccult and HemoccultSensa sensitivities of 11% (CI, 6% to 16%) and 21% (CI, 15% to 27%), respectively (Table 3). Table 4 shows the test positivity rates for subgroups of colorectal lesions.

For adenomas 1 cm or larger, the positivity rate was 46% (CI, 35% to 54%) by SDT-2 versus 10% (CI, 4% to 15%) by Hemoccult ($P < 0.001$) and 17% (CI, 9% to 24%) by HemoccultSensa ($P < 0.001$). Neoplasm site did not affect detection by SDT-2; however, detection rates were lower for lesions proximal to the splenic flexure than for distal lesions with both Hemoccult (9% [CI, 1% to 16%] vs. 21% [CI, 12% to 30%]; $P = 0.060$) and HemoccultSensa (13% [CI, 4% to 21%] vs. 31% [CI, 22% to 41%]; $P = 0.010$). Study comparisons are based on a single stool per patient for SDT-2 and 3 stools per patient for fecal blood tests; differences in test performance are larger if fecal blood test results are analyzed on fewer than 3 stools per patient (Figure 2). For patients with normal colonoscopy results, the positivity rate was 16% (CI, 8% to 24%) by SDT-2, compared with 4% (CI, 1% to 11%) by Hemoccult ($P = 0.010$) and 5% (CI, 1% to 13%) by Hemoccult-Sensa ($P = 0.030$).

Frequencies of DNA Markers in Neoplastic Tissue

Nearly all of the tissues analyzed from screen-relevant neoplasms contained at least 1 marker from the SDT-2 panel; fewer than two thirds of the tissues contained SDT-1 markers (Table 5).

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Negative Stool DNA Tests in Patients with Screen-Relevant Neoplasia

Incomplete marker recovery from stools, instability of long DNA, and lesion size influenced test results. Recovery of individual markers in stool when they were present in tumor tissue from the same patient was 40% (17 of 42) for SDT-1 and 39% (51 of 130) for SDT-2. The sensitivity of long DNA decreased as the time from defecation to freezing the stool increased; sensitivity for screen-relevant neoplasms decreased from 11% on stools received and frozen 12 to 24 hours after defecation to 1.8% at 24 to 35 hours and to 0% at more than 35 hours after defecation ($P = 0.010$). The sensitivity of mutation markers did not change over this time range. The median size of screen-relevant neoplasms missed by SDT-2 was 15 mm, compared with 20 mm for detected lesions ($P = 0.003$). Neither demographic variables nor randomization group affected stool DNA test positivity.

Positive Stool DNA Tests in Patients without Screen-Relevant Neoplasia

Diet and use of nonsteroidal anti-inflammatory drugs affected HemoccultSensa results but not stool DNA test or Hemoccult results. For HemoccultSensa, positivity rates in colonoscopically normal patients randomly assigned to group A (restricted diet and medications) were 2% (CI, 1% to 2%), compared with 4% (CI, 3% to 5%) for those in group B (unrestricted) ($P = 0.030$). In contrast, positivity rates for the 2 stool DNA tests were unaffected (SDT-1: 4% for group A vs. 3% for group B [$P = 0.31$]; SDT-2: 19% for group A vs. 13% for group B [$P = 0.50$]), as were those for Hemoccult (1% for group A vs. 2% for group B; $P = 0.170$). Age influenced SDT-2 results in patients with normal colonoscopy results, as positivity rates increased from 6% (CI, 4% to 29%) for patients younger than age 65 years to 26% (CI, 12% to 39%) for those 65 years of age or older ($P = 0.020$) (Table 4). Positivity rates for the component markers of SDT-2 were 6% and 15% for *APC* scanning, 0% and 8% for methylated vimentin, and 0% and 3% for mutant *K-ras* in patients younger than age 65 years and those 65 years of age or older, respectively. Other demographic factors did not affect stool DNA test positivity.

Comparison of Stool DNA Tests

We performed the 2 stool DNA tests on 69 patients with screen-relevant neoplasia and 54 with normal colonoscopy results. Positivity rates were higher with SDT-2 than with SDT-1 among the 69 patients with screen-relevant neoplasms (43% [CI, 32% to 55%] vs. 20% [11% to 30%]; $P = 0.001$), including the subset of 12 patients with colorectal cancer (58% [CI, 28% to 85%] vs. 25% [6% to 57%]; $P = 0.050$). Positivity rates were higher when comparing results for all 47 patients with adenomas 1 cm or larger (45% [CI, 31% to 59%] vs. 13% [CI, 3% to 22%]; $P = 0.003$), the 21 patients with adenomas larger than 2 cm (62% [CI, 38% to 82%] vs. 19% [CI, 5% to 42%]; $P = 0.003$), and the 26 patients with adenomas between 1 and 2 cm (31% [CI, 14% to 52%] vs. 8% [CI, 1% to 25%]; $P = 0.030$). Test positivity in those with normal colonoscopy results was significantly higher with SDT-2 than with SDT-1 (13% [CI, 5% to 25%] vs. 2% [CI, 1% to 10%]; $P = 0.030$).

Discussion

In this multicenter study, we found that the precommercial stool DNA test (SDT-1) provides no meaningful improvements over the widely used fecal occult blood tests Hemoccult and

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HemoccultSensa for detection of screen-relevant colorectal neoplasms. Neoplasm detection rates by SDT-1 were no better than HemoccultSensa for any subset of neoplasms; in fact, HemoccultSensa detected significantly more cases of cancer than did SDT-1. In addition, the estimated positive likelihood ratios for cancer or high-grade dysplasia tended to be larger and the negative likelihood ratios smaller with both fecal blood tests than with SDT-1.

Because of the poor performance of SDT-1, 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. Whereas fecal blood testing detected proportionately fewer proximal than distal colorectal neoplasms, corroborating previous findings (26, 27), site did not affect detection by stool DNA testing.

Detection of adenomas is essential for screening to prevent colorectal cancer. In our blinded comparison, using colonoscopy results as the criterion standard, SDT-2 detected 3 times more adenomas that were 1 cm or larger than did SDT-1, 4 times more than did Hemoccult and 3 times more than did HemoccultSensa. Compared with SDT-1, all markers in the SDT-2 panel occur early in the adenoma-to-cancer progression (25, 28, 29) and were collectively more informative for adenomas on our tissue analyses; this accounted for the superior detection rates of SDT-2 over SDT-1. Our finding that guaiac testing is insensitive for detecting adenomas is consistent with previous studies in the screening setting (21, 26, 30, 31). We 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 (32–34). Although most adenomas seem not to bleed, our data suggest that many do shed molecular markers that can be detected in stool.

Technical advances could improve DNA assay sensitivity. Although we found SDT-2 markers in tissue from nearly all screen-relevant neoplasms, the assay detected the markers in stools from the same patient in fewer than one half of cases. Methods to increase stool DNA capture are needed, and some have been reported (20, 24). Preservation of intact DNA sequences in stool could also increase marker recovery. Although long DNA was the most informative panel marker in earlier studies done with minimal processing delays (12, 15, 16), we observed that long DNA degraded during shipment to the study coordinating center and contributed little to neoplasm detection. We (35) and others (36) have shown that adding a preservative to stool at the time of defecation can prevent degradation of long DNA. Finally, because DNA markers are present in such low levels (9), augmented technical sensitivity may further enhance lesion detection (37).

Test positivity among colonoscopically normal patients was significantly more frequent with SDT-2 than with Hemoccult or HemoccultSensa, probably because of the disproportionately elevated positivity rates in older patients; however, the sample size was small and the confidence intervals were relatively wide. False-positive rates for the SDT-2, Hemoccult, and HemoccultSensa tests were similar among participants younger than 65 years of age. Other studies have reported that *K-ras* mutations (38) and methylated vimentin (20) in stool increase with age. Accumulated DNA alterations may precede endoscopically visible lesions

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and account for such age-related apparent false-positives. For example, aberrant crypt foci—putative neoplasm precursors not seen by conventional colonoscopy—increase with age (39), and mutant *K-ras* (39, 40), mutant *APC* (40), and gene methylation (28, 41) have all been reported in aberrant crypt foci. Whether molecular detection of such preneoplastic field changes predicts future cancer risk is not known.

Our study differs from the only other multicenter study of stool DNA testing in average-risk patients (21) in several important ways. First, unlike the earlier report (21), we did not find that SDT-1 was better than Hemoccult for detection of invasive cancer. Second, we followed a standardized approach in a central laboratory for guaiac testing, which may account for our much higher Hemoccult sensitivity for cancer. Others have shown that central laboratory testing is more accurate than office testing after digital rectal examination (42, 43), but we do not know whether results from patient-smeared stools are more accurate when processed in a centralized laboratory rather than in a physician's office. Our study also included the more sensitive HemoccultSensa test. Third, all pathology was reviewed centrally and neoplasm tissue was procured for DNA analyses, allowing us to measure marker recovery rates in the stool. Fourth, our participants were randomly assigned, which demonstrated that neither diet nor nonsteroidal anti-inflammatory drug use affected stool DNA results. Finally, the previous study did not evaluate SDT-2.

Our study provides robust data on the accuracy of guaiac-based occult blood tests in the screening setting. Published results vary widely according to testing algorithms and patient populations, and our Hemoccult results fall in reported ranges from studies done in the screening setting, as reviewed (44). Both Hemoccult and Hemoccult-Sensa achieved high specificity, regardless of diet, on tests performed according to the manufacturer's instructions and in a centralized laboratory. HemoccultSensa was more sensitive than Hemoccult for all types of screen-relevant neoplasms, and HemoccultSensa detected more than 60% of cases of cancer. However, both tests missed the large majority of the adenomas that were 1 cm or larger. Such low detection rates of premalignant adenomas may account for the reported modest (45) or absent (3, 4, 46) effect of long-term Hemoccult screening on cancer incidence.

Our study has limitations. We did not test all participants or subsets with SDT-2, and those without screen-relevant neoplasm were particularly underrepresented. We used SDT-2 to test samples from all patients with cancer, high-grade dysplasias, or adenomas larger than 2 cm; 50 randomly selected samples from patients who had adenomas measuring between 1 and 2 cm; and 75 randomly selected samples from patients with normal colonoscopy results. We did not test samples from patients with small adenomas or hyperplastic polyps by using SDT-2, and their inclusion may have further increased false-positive rates. In addition, stabilization buffer was not added to stools at time of collection. Use of a stabilization buffer may have improved detection of long DNA and possibly other DNA markers as well.

In summary, stool DNA testing by SDT-1 offered no clear advantages over fecal occult blood testing for screening detection of colorectal neoplasia. Stool DNA test 2, which targets more broadly informative markers, detected significantly more screen-relevant neoplasms than did Hemoccult or HemoccultSensa, and detection was especially improved for

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adenomas. However, we do not know whether this gain in sensitivity is offset by a loss in specificity. Molecular technology will continue to evolve and provide opportunities for needed assay refinements in stool DNA testing.

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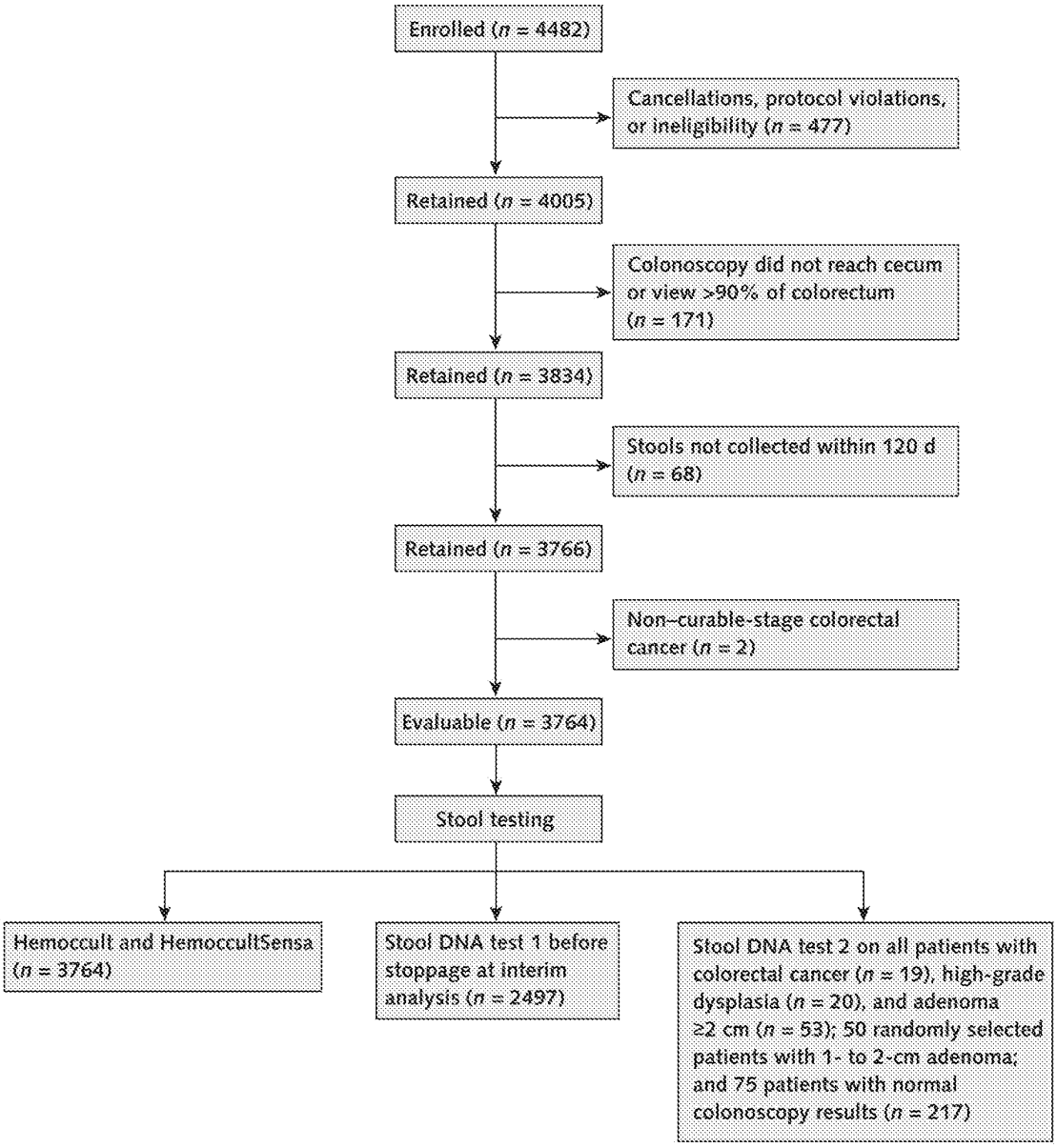


Figure 1. Study flow diagram

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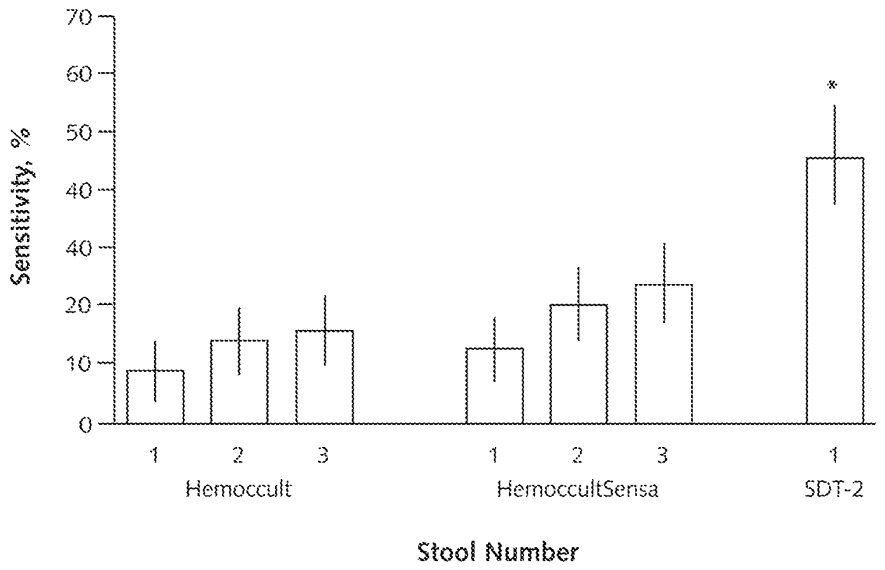


Figure 2. Stool DNA versus occult blood testing for detection of screen-relevant neoplasia (curable-stage colorectal cancer, high-grade dysplasia, and adenomas ≥1 cm) (n = 142)

Sensitivity is plotted for Hemoccult and HemoccultSensa with 1, 2, and 3 stools per patient and with stool DNA test 2 (*SDT-2*) on a single stool per patient. Vertical lines represent 95% CIs.

*$P < 0.001$ versus Hemoccult or HemoccultSensa.

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Table 1

Definitions

Gene targets in stool DNA test panels:
Test 1: point mutations on *K-ras*, *APC*, and *p53*; microsatellite marker BAT-26; long DNA
Test 2: point mutations on *K-ras*, scanned mutator cluster region of *APC*, vimentin methylation
Screen-relevant neoplasia: colorectal cancer, high-grade dysplasia, adenomas ≥1 cm
Sensitivity: rate of test positivity for those with screen-relevant neoplasia
Specificity: rate of test negativity for those without screen-relevant neoplasia
Test positivity: rate of positive stool test results for individual colonoscopic findings or groups of findings

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Table 2

Baseline Characteristics and Colorectal Findings

Characteristic	All Patients (n = 4482)	Evaluable Patients* (n = 3764)	Patients Tested with SDT-1† (n = 2497)	Patients Tested with SDT-2‡ (n = 217)
Age, *y*				
Mean (SD)	63.8 (8.29)	63.7 (8.25)	60.4 (7.86)	66.4 (7.17)
Median (range)	65 (50–81)	65 (50–80)	59 (50–80)	67 (51–80)
Women, *n (%)*	2341 (52.2)	1964 (52.2)	1348 (54.0)	108 (49.8)
White, *n (%)*	4184 (93.4)	3522 (93.6)	2314 (92.7)	201 (92.6)
Colorectal findings, *n (%)*				
Screen-relevant neoplasia	–	290 (7.7)	157 (6.3)	142 (65.4)
Cancer				
Stage I	–	11 (0.3)	6 (0.2)	11 (5.1)
Stages II and III	–	8 (0.2)	6 (0.2)	8 (3.7)
Cancer + high-grade dysplasia	–	39 (1.0)	22 (0.9)	39 (18.0)
Adenoma ≥1 cm	–	251 (6.7)	135 (5.4)	103 (47.5)
Adenoma >2 cm	–	53 (1.4)	21 (0.8)	53 (24.4)
Adenoma <1 cm	–	785 (20.9)	469 (18.8)	Not tested
Hyperplastic polyps	–	492 (13.1)	341 (13.7)	Not tested
Other	–	86 (2.3)	57 (2.3)	Not tested
Normal	–	2111 (56.1)	1473 (59.0)	75 (34.6)

SDT = stool DNA test.

*Patients who met all inclusion criteria. Both Hemoccult and HemoccultSensa (Beckman Coulter, Fullerton, California) were performed on all evaluable participants.

†On the basis of results from an interim analysis, SDT-1 was terminated.

‡All participants with cancer, high-grade dysplasia, and adenomas ≥2 cm from the full enrollment period are included, as are random samples from 50 patients with 1- or 2-cm adenomas and 75 with normal colonoscopy results.

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Table 3

Summary of Test Performance

Index Test	Screen-Relevant Neoplasia, *n* *	Positive Test Result, *n*	Sensitivity (95% CI)	No Screen-Relevant Neoplasia, *n*	Negative Test Result, *n*	Specificity (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Hemoccult (*n* = 2497)	157	17	11 (6–16)†	2340	2297	98 (98–99)†	5.9 (3–10)	0.9 (0.9–1.0)
HemoccultSensa (*n* = 2497)	157	33	21 (15–27)§	2340	2258	97 (96–97)‖	6.0 (4–9)	0.8 (0.8–0.9)
SDT-1 (*n* = 2497)	157	31	20 (14–26)	2340	2246	96 (95–97)	4.9 (3–7)	0.8 (0.8–0.9)
SDT-2 (*n* = 217)	142	66	40 (32–49)¶	75	NA**	NA	NA	NA

NA = not available; SDT = stool DNA test.

* Includes curable-stage cancer, high-grade dysplasia, and adenomas ≥1 cm.

† $P = 0.02$ for STD-1 vs. Hemoccult.

‡ $P < 0.001$ for STD-1 vs. Hemoccult.

§ $P = 0.80$ for STD-1 vs. HemoccultSensa.

‖ $P = 0.40$ for STD-1 vs. HemoccultSensa.

¶ We calculated the weighted sensitivity for SDT-2 with the following equation:

$$\text{reweighted sensitivity} = (\%\,[\text{colorectal cancer} + \text{high-grade dysplasia}] \times \text{PR}) + (\%\,\text{adenomas} \geq 2\,\text{cm} \times \text{PR}) + (\%\,\text{adenomas}\ 1\text{–}2\,\text{cm} \times \text{PR}) = (0.13 \times 0.49) + (0.18 \times 0.57) + (0.68 \times 0.34)$$

PR = proportion of participants for that category of screen-relevant neoplasia in the entire population with screen-relevant neoplasia. See "Comparison of Stool DNA Tests" for statistical comparisons of SDT-1 and SDT-2 in participants who had both DNA tests performed.

** We did not calculate formal specificity because SDT-2 was not performed on all subsets without screen-relevant neoplasia.

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Table 4

Test Positivity Rates

Stool DNA Test and Patient Subset	Positive SDT Result (95% CI), %	Positive Hemoccult Result (95% CI), %	*P* Value*	Positive HemoccultSensa Result (95% CI), %	*P* Value†
SDT-1					
Screen-relevant neoplasms‡ (*n* = 157)	20 (14–26)	11 (6–16)	0.020	21 (15–27)	0.80
Site					
Proximal§ (*n* = 56)	20 (9–30)	7 (2–17)	0.070	11 (3–19)	0.20
Distal§ (*n* = 101)	20 (12–28)	13 (6–19)	0.100	27 (18–35)	0.20
Cancer (*n* = 12)	25 (5–57)	50 (22–78)	0.30	75 (51–100)	0.019
Cancer + high-grade dysplasia (*n* = 22)	36 (16–56)	41 (20–61)	0.80	55 (34–75)	0.20
Adenoma ≥1 cm‖ (*n* = 135)	17 (11–23)	6 (2–10)	0.004	16 (9–22)	0.74
Adenoma >2 cm (*n* = 21)	19 (5–42)	14 (3–36)¶	0.60	33 (13–54)	0.30
Adenoma 1–2 cm (*n* = 114)	17 (10–24)	4 (1–8)¶	0.004	12 (6–18)	0.40
Adenoma <1 cm (*n* = 469)	4 (2–6)	3 (1–5)	0.30	5 (3–7)	0.70
Hyperplastic polyps (*n* = 341)	5 (2–7)	1 (0.2–3)¶	0.003	4 (2–6)	0.70
Other (*n* = 57)	5 (1–15)	2 (0.04–9)	0.30	5 (1–15)#	1.00
Normal (*n* = 1475)	4 (3–5)	2 (1–2)	<0.001	3 (2–4)	0.20
Age <65 y (*n* = 1040)	3 (2–4)	2 (1–2)	0.009	3 (2–4)	0.40
Age >65 y (*n* = 433)	5 (3–7)	2 (1–3)	0.020	3 (2–5)	0.30
SDT-2					
Screen-relevant neoplasms‡ (*n* = 142)	46 (38–55)	16 (10–22)	<0.001	24 (17–31)	<0.001
Site					

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Stool DNA Test and Patient Subset	Positive SDT Result (95% CI), %	Positive Hemoccult Result (95% CI), %	*P* Value*	Positive HemoccultSensa Result (95% CI), %	*P* Value†
Proximal§ (*n* = 56)	45 (32–58)	9 (1–16)	<0.001	13 (4–21)	<0.001
Distal§ (*n* = 86)	48 (37–58)	21 (12–30)	<0.001	31 (22–41)	0.020
Cancer (*n* = 19)	58 (36–80)	47 (25–70)	0.40	63 (41–85)	0.70
Cancer + high-grade dysplasia (*n* = 39)	49 (33–64)	33 (19–48)	0.100	44 (28–59)	0.60
Adenoma ≥ 1 cm (*n* = 103)	46 (35–54)	10 (4–15)	<0.001	17 (9–24)	<0.001
Adenoma > 2 cm (*n* = 53)	57 (43–70)	13 (4–22)	<0.001	25 (13–36)	<0.001
Adenoma 1–2 cm (*n* = 50)	34 (21–47)	6 (1–17)‖	0.002	8 (2–19)‖	0.005
Normal (*n* = 75)	16 (8–24)	4 (1–11)‖	0.010	5 (1–13)‖	0.030
Age <65 y (*n* = 36)	6 (4–29)‖	3 (0–15)‖	0.56	6 (1–19)‖	1.00
Age >65 y (*n* = 39)	26 (12–39)	5 (1–17)‖	0.010	5 (1–17)‖	0.010

SDT = stool DNA test.

*For SDT versus Hemoccult.

†For SDT versus HemoccultSensa.

‡Patients were classified by the most advanced lesion found (in descending order of severity: cancer, high-grade dysplasia, or adenoma). The distribution of screen-relevant neoplasms differs from that in Table 3. See Table 2 for details of patients tested with SDT-2.

§Proximal and distal sites are relative to the splenic flexure. "Proximal" includes the cecum and ascending and transverse colon, "distal" includes the splenic flexure, descending colon, sigmoid, and rectum.

‖The exact CI was used when the numerator was <5.

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Table 5

Presence of DNA Markers in Tumor Tissue*

Marker	SDT-1 Panel						SDT-2 Panel				
	Patients, *n*	*K-ras*, %	*APC*†, %	*p53*, %	BAT-26, %	Full Panel, %	Patients, *n*	*K-ras*, %	*APC*‡, %	Vimentin, %	Full Panel, %
Cancer and high-grade dysplasia	20	45	35	25	0	60	35	51	60	63	94
Adenoma ≥1 cm	48	42	38	6	2	63	99	39	73	63	98
All screen-relevant neoplasms§	68	43	37	12	1	62	134	43	69	63	97

SDT = stool DNA test.

*Tissues were analyzed from all available samples from patients with cancer and high-grade dysplasia and from a random sample of patients with adenomas ≥1 cm.

†The assay targeted 10 high-frequency mutations on the *APC* gene by using a single-base extension method (see citations in Methods).

‡The assay targeted any mutation in the mutator cluster region of the *APC* gene by using the scanning method (see citations in Methods).

§Includes curable-stage cancer, high-grade dysplasia, and adenomas ≥1 cm.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	*Ex Parte* Reexamination of U.S. Patent No. 11,634,781
Issued:	April 25, 2023
App. Ser. No.:	17/936,335
Filing Date:	September 28, 2022
Named Inventor:	Joost Louwagie
Title:	FECAL SAMPLE PROCESSING AND ANALYSIS COMPRISING DETECTION OF BLOOD

Mail Stop: *Ex Parte* Reexam
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MR. ANTHONY P. SHUBER, MS

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I, Mr. Anthony P. Shuber, MS, declare and state as follows:

1. I am submitting this declaration in connection with a Request for *Ex Parte* Reexamination of U.S. Patent No. 11,634,781 ("the '781 Patent"). I have been asked by the Requester to provide information and opinions regarding the level of skill of a person of ordinary skill in the art for the invention claimed in the '781 Patent, the general knowledge of such a person at the time of the effective filing date for the '781 Patent, the scope and content of the prior art references, whether the invention claimed in the '781 Patent was previously described by the prior art, and whether the invention claimed in the '781 Patent would have been obvious to a person of ordinary skill in the art in view of the prior art.

2. For my work on this case I am charging my customary consulting rate. My opinions and work on this matter are not dependent on the outcome of this matter.

I. RELEVANT EXPERIENCE

3. As described in my *curriculum vitae*, which is attached hereto, my research interests include developing Molecular Diagnostic Assays applied to both inherited diseases and cancer.

4. In 1980, I received a Bachelor of Science degree in Biology from Marquette University in Milwaukee, Wisconsin. Thereafter, in 1983, I received a Master of Science degree in Molecular and Developmental Biology from Marquette University in Milwaukee, Wisconsin.

5. My prior work in the field includes developing several DNA diagnostics tests, while employed at Genzyme Genetics. One of those tests involved a novel and proprietary approach to multi-plex and analyze hundreds of inherited disease diagnostic mutations that spanned across multiple diseases such as Cystic Fibrosis, Sickle Cell Anemia, and several others. Subsequent to my work in developing molecular diagnostic assays for inherited disorders, I focused the majority of my efforts on developing diagnostics in oncology, with my major area of interest involving the early detection of cancer.

6. Currently, I am employed as Chief Innovation Officer at Harbinger Health.

7. My research interests include developing and creating novel diagnostic assays for the early detection of cancer as well as novel approaches to cancer treatment. In my career, I authored or co-authored over 35 journal articles on diagnostics methodologies that have been

published in peer-reviewed journals. Furthermore, I am a named inventor or co-inventor on at least 100 patents or patent applications.

II. DOCUMENTS AND INFORMATION CONSIDERED

8. In providing the information and opinions in this declaration, I have reviewed and relied upon the documents listed below, each of the other documents referred to in this declaration, and the documents that are attached as exhibits to this declaration.

Ex. No.	Prior Art Citation
1001	U.S. Patent No. 11,634,781 ("the '781 Patent")
1002	U.S. Patent No. 5,952,178 to Lapidus et al. ("Lapidus '178")
1003	U.S. Patent No. 5,741,650 to Lapidus et al. ("Lapidus '650")
1004	Ahlquist et al., "Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia," *Ann Intern Med.*, 149(7):441-W81 (published in final form October 7, 2008) ("Ahlquist")
1005	Patent Cooperation Treaty (PCT) Publication No. WO 2005/014154 to O'Neill ("O'Neill")
1006	European Patent Application Publication No. EP1366715A1 to De Luca et al. ("De Luca")
1007	Olson et al., "DNA Stabilization Is Critical for Maximizing Performance of Fecal DNA-Based Colorectal Cancer Tests," *Diagn Mol Pathol*, 14:183–191 (published September 2005) ("Olson")
1008	Leung et al., "Detection of Hypermethylated DNA or Cyclooxygenase-2 Messenger RNA in Fecal Samples of Patients With Colorectal Cancer or Polyps," *Am J Gastroenterol*, 102:1070–1076 (2007) ("Leung")
1009	Hoepffner et al., "Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study," *Aliment Pharmacol Ther*, 23, 145–154 (published 2006) ("Hoepffner")
1010	U.S. Patent No. 4,859,610 to Maggio ("Maggio")
1011	U.S. Patent No. 5,196,167 to Guadagno et al. ("Guadagno")
1012	U.S. Patent No. 5,137,806 to LeMaistre et al. ("LeMaistre")
1013	U.S. Patent No. 5,527,676 to Vogelstein et al. ("Vogelstein")

9. In addition to the materials above, I relied upon my education and experience, as detailed above and in my attached *curriculum vitae*, to provide the information and opinions in this declaration.

III. APPLICABLE LEGAL STANDARDS

10. I understand that the issues presented in this *ex parte* reexamination must be considered in view of certain applicable legal standards. I am not a lawyer. However, the following is a summary of my general understanding of certain legal principles and standards that I have used in forming my opinions expressed below, including, in particular, my general understanding of the legal concepts of "anticipation" and "obviousness."

11. I understand that 35 U.S.C. § 102 ("§ 102") provides the standard for determining whether a claim in a patent is invalid as anticipated ("not novel"). I understand that a claim in a patent is invalid as anticipated under §102 when each and every element of the claim is described in a single prior art reference as understood by a person of ordinary skill in the art. I understand that the only grounds for anticipation that may be presented in a request for *ex partes* reexamination are those based on patents and/or printed publications. I further understand that for *ex parte* reexamination, anticipation must be proven by a preponderance of the evidence, which I understand to mean "more likely than not."

12. I understand that 35 U.S.C. § 103 ("§ 103") provides the standard for determining whether a claim in a patent is invalid as obvious. I understand that a claim in a patent is invalid as obvious under § 103 when the differences between the claimed subject matter and the prior art are such that the claimed subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art. I understand that the only grounds for obviousness that may be presented in a request for *ex partes* reexamination are those based on patents and/or printed publications. I further understand that for *ex partes* reexamination, obviousness must be proven by a preponderance of the evidence, which I understand to mean "more likely than not."

13. I understand that, in conducting the obviousness analysis, it is important to identify a reason that would have prompted a person of ordinary skill in the art to combine prior art references in a way that would have rendered the claimed invention obvious and that the person would have had a reasonable expectation of success in making the claimed invention. I

also understand that whether or not it would have been obvious to combine or modify prior art may depend in part on whether such a person would have found the claimed invention to be a predictable use of prior art elements according to their established functions.

14. I understand that a prior art reference may be considered in assessing a patent claim's obviousness if the reference discloses solving any problem or addressing any need addressed by the patent, or if the reference discloses information having obvious uses beyond its primary purpose that a person of ordinary skill in the art would reasonably examine to solve a problem or address a need presented by the patent. I understand that a claimed combination of familiar elements using known methods is likely obvious when it does no more than yield predictable results. I understand that when a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same or a different field. I understand that if a person of ordinary skill in the art can implement a predictable variation of a claimed invention, then the claimed invention is likely obvious.

15. I understand that an obviousness analysis need not seek out precise teachings directed to the specific subject matter of the claimed invention, and that the Patent Office can take account of the inferences and creative steps that a person of ordinary skill in the art would have employed at the time of the alleged invention. I understand that it is often necessary to look to interrelated teachings of multiple prior art references; the effects of demands known to those working in the relevant field of art or present in the marketplace; and the background knowledge possessed by a person of ordinary skill in the art, all in order to determine whether there was an apparent reason for the person of ordinary skill in the art to have combined elements in the manner claimed. I understand that any need or problem known in the field of endeavor at the time of the alleged invention or addressed by the patent-at-issue can provide a reason for the person of ordinary skill in the art to have combined elements in the manner claimed. I understand that one way in which a patent's subject matter can be proved obvious is by noting that there existed at the time of invention a known problem for which there was an obvious solution encompassed by the patent's claims. I understand that when there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp. I further understand that, if this leads to the anticipated success, it is likely the result not of innovation but of ordinary skill and common sense and, as such, likely is obvious.

16. I also understand that in determining the meaning of claim terms in a patent, the claim terms of patents are given the broadest reasonable interpretation consistent with the specification and that limitations in the specification are not read into the claims.

17. Detailed explanations of my opinions in this matter as well as the basis for each of my opinions are set forth below. I reserve the right to supplement my opinions based on any new information that is provided to me in this matter.

IV. QUALIFICATIONS AND KNOWLEDGE OF A PERSON OF ORDINARY SKILL IN THE ART

18. It is my opinion that the relevant field of the invention of the '781 Patent is the field of processing and evaluating fecal samples.

19. I understand that the knowledge level of one with ordinary skill in the art is determined by considering various factors, including the type of problems encountered in the art; prior art solutions to those problems; rapidity with which innovations are made; sophistication of the technology; and educational level of active workers in the field. I understand that the earliest possible effective filing date that could be accorded the '781 Patent is February 3. 2009. Solely for the purpose of this declaration, I have been asked to assume that February 3, 2009, is the effective filing date of each of the claims of the '781 Patent and to address the ordinary level of skill in the art at that time.

20. Taking into account the above factors, and based on my experience in this field and with persons working in this field, it is my opinion that a person of ordinary skill in the art of the '781 Patent as of February 3. 2009, would have had at least a Bachelor of Science degree in Biology (or a similar major) and several years of experience in the processing of biological samples, such as fecal samples from a human. Such a person would have been well-acquainted with methods of collecting fecal samples, preserving proteins and/or nucleic acids, including hemoglobin, DNA, and RNA, and subsequently evaluating the fecal samples for proteins and/or nucleic acids, including hemoglobin, DNA, and RNA, as of February 3, 2009.

21. I currently possess the level of ordinary skill in the art of the '781 Patent. I also possessed that level of skill as of February 3, 2009. Furthermore, I possessed that level of skill at the time I co-invented subject matter described and claimed in the Lapidus '178 and Lapidus

'650 references (further discussed below). All of my opinions in this declaration are from the perspective of a person of ordinary skill in this art of the '781 Patent as of February 3. 2009.

V. SUMMARY OF THE '781 PATENT

22. I have reviewed the claims and specification for the '781 Patent and provide the following summary of the claimed invention of the '781 Patent. A copy of the '781 Patent is attached to the Request as Exhibit 1001.

23. The '781 Patent generally describes methods of processing a fecal sample from a human subject. Ex. 1001 at Abstract. The methods of the '781 Patent involve collecting a fecal sample, removing a portion of a collected fecal sample and combining the removed portion with a buffer that prevents denaturation or degradation of blood proteins, and combining a buffer with the remaining portion of the fecal sample. *Id.*

24. The '781 Patent includes one independent claim as explained below.

VI. CLAIM TERMS IN THE '781 PATENT

25. I have reviewed the claims and specification for the '781 Patent and provide the following comments on certain terms found in the claims of the '781 Patent. A copy of the '781 Patent is attached to the Request as Exhibit 1001.

26. My opinions regarding the meaning of claim terms of the '781 Patent are based on the plain and ordinary meanings of the claim terms in the context of the entire '781 Patent, including its specification and prosecution history.

27. The claims of the '781 Patent require that a fecal sample is processed "without freezing." The ordinary meaning of "freezing" is being at or below the temperature at which water freezes and the temperature at which water freezes is known to be 0°C (32°F). Thus, a person of ordinary skill in the art would understand "without freezing" to include any temperature above 0°C (32°F) or a temperature range that includes a temperature above 0°C (32°F).

28. The claims of the '781 Patent require that a fecal sample is collected "at home." The '781 Patent uses this term primarily to distinguish the location of collection from the testing facility. For instance, the '781 Patent states that "[f]ollowing collection of the sample and suitable processing, the method may further comprise forwarding or otherwise delivering the

removed portion of the collected faecal sample *to a laboratory* for performing step (a) of the method, as defined herein, on the removed portion of the collected faecal sample." *Id.* at column 18, lines 32-35 (emphasis added). Similarly, dependent claim 2 of the '781 Patent indicates that the method of claim 1 *further comprises* "delivering the [fecal sample collected at home] to a medical diagnostics laboratory." *Id.* at column 45, lines 39-44. Therefore, a person of ordinary skill in the art would understand "at home," as used in the '781 Patent, to refer to a location other than the testing facility.

29. 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." *Id.* at column 12, lines 35-39. A skilled person would understand the '781 Patent's definition of "epigenetic modification" as the plain and ordinary meaning of the term.

VII. COMPARISON OF CLAIMS OF THE '781 PATENT WITH PRIOR ART REFERENCES

30. I have been asked to review various prior art references that relate to the claimed invention of the '781 Patent, including Lapidus '178 and Lapidus '650. I am one of the named inventors of both Lapidus '178 and Lapidus '650 and, thus, am very familiar with their contents.

31. I understand that Lapidus '178 is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was patented on September 14, 1999, more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

32. I understand that Lapidus '650 is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was patented on April 21, 1998, more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

33. As discussed in detail below, it is my opinion that the description of Lapidus '178 and the description of Lapidus '650 each anticipate claims 1-4 and 12 of the '781 Patent and also render claims 1-4 and 12 of the '781 Patent obvious.

34. I have also reviewed and relied on Maggio as evidencing certain aspects of the disclosure of Lapidus '178 and Lapidus '650. I understand that Maggio is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was patented on August 22, 1989, more than

one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

35. I have also reviewed and relied upon the disclosures of Guadagno, LeMaistre, and Vogelstein, each of which are incorporated by referenced into both Lapidus '178 and Lapidus '650. Furthermore, I understand that Guadagno, LeMaistre, and Vogelstein are also themselves prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because they were each patented more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

36. I also reviewed the disclosures of Ahlquist, O'Neill, De Luca, Olson, Leung, and Hoepffner.

37. I understand that Ahlquist is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(a) because it was published on October 7, 2008, and reasonably accessible to the public interested in the art before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

38. I understand that O'Neill is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was published on February 17, 2005, more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

39. I understand that De Luca is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was published on December 3, 2003, more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

40. I understand that Olson is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was published in September 2005, more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

41. I understand that Leung is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was published in 2007, and reasonably accessible to the public interested in the art more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

42. I understand that Hoepffner is prior art to the '781 Patent under pre-AIA 35 U.S.C. § 102(b) because it was published in 2006, and reasonably accessible to the public interested in the art more than one year before the earliest possible effective filing date for the claims of the '781 Patent (February 3, 2009).

43. As discussed in detail below, it is my opinion that the teachings of Lapidus '178, Lapidus '650, Ahlquist, or O'Neill, in combination with the teachings of one another and/or with De Luca, Olson, Leung, and/or Hoepffner, render claims 1-20 of the '781 Patent obvious. It is also my opinion, discussed below, that a person of ordinary skill in the art as of February 3, 2009 would have been motivated to combine Lapidus '178, Lapidus '650, Ahlquist, O'Neill, De Luca, Olson, Leung, and/or Hoepffner with a reasonable expectation of success.

A. Claim 1 Was Anticipated and Obvious

1) Preamble of Claim 1

44. The preamble of claim 1 of the '781 Patent provides "[a] method of processing a freshly-collected fecal sample without freezing, the method comprising…". As stated above, a person of ordinary skill in the art would understand that "without freezing" includes any temperature above 0°C (32°F), or a temperature range that includes a temperature above 0°C (32°F). Lapidus '178, Lapidus '650, and Ahlquist all teach methods of processing freshly-collected fecal samples without freezing.

45. Lapidus '178 describes a freshly-collected stool (a fecal sample) voided by a patient into a receptacle that is transported to a testing facility. Ex. 1002 at column 7, lines 58-64. Lapidus '178 states that, in "a preferred embodiment, a stool is frozen prior to processing." *Id.* at column 9, lines 21-22. This statement reflects that the inventors (Mr. Lapidus and I) also contemplated non-preferred embodiments in which the stool was not frozen prior to processing. As such, Lapidus '178 discloses this feature of claim 1.

46. Lapidus '650 teaches processing of freshly-collected stool samples and does not teach freezing those samples. Ex. 1003 at column 7, lines 12-17; *id.* at column 7, lines 24-26. Additionally, Lapidus '650 (at column 6, lines 30-31) incorporates by reference U.S. Patent No. 5,196,167 ("Guadagno"), which discloses a test sheet product used for identification of blood in a patient stool and states that "[a]fter the patient has completed a bowel movement, the test sheet is merely dropped into the toilet bowl." Ex. 1011 at column 6, lines 65-67. Guadagno further discloses that "[a]fter the results of the test have been visually observed, the test sheet is disposed of by merely flushing the toilet bowl." *Id.* at column 7, lines 7-9. Accordingly, a person of ordinary skill in the art would recognize Lapidus '650 discloses the method of processing a "freshly-collected" fecal sample "without freezing."

47. Furthermore, any minor differences that may exist between the preamble of claim 1 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would be used to process freshly-collected fecal samples without freezing those samples. Thus, at a minimum, this aspect of claim 1 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

48. Ahlquist also teaches methods of processing freshly-collected fecal samples without freezing. Ex. 1004 at page 4, at 'Stool Collection and Processing' heading. 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. *Id.* Further, Ahlquist indicates that the stools were frozen after they were received at the Mayo Clinic. *Id.* This indicates to a person skilled in the art that the freshly-collected fecal samples were processed by the patients without freezing in Ahlquist. As such, Ahlquist teaches the preamble of claim 1.

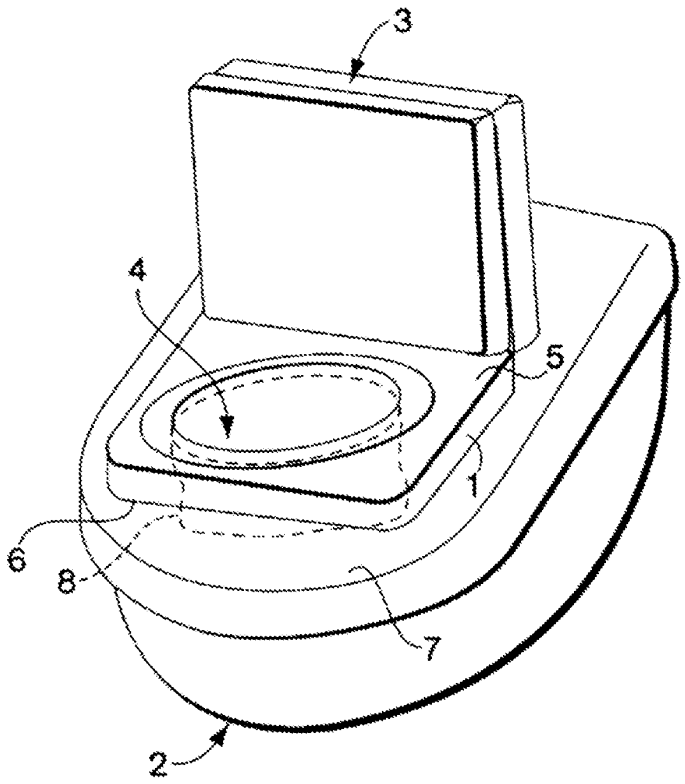
49. O'Neill also teaches methods of processing freshly-collected fecal samples without freezing. Ex. 1005 at page 6, lines 14 to page 8, line 11. O'Neill discloses collection of stool (a fecal sample) into a container. *Id.* Although O'Neill suggests that the stools can be cooled to a temperature below its "gel freezing" point, O'Neill teaches that this temperature range includes temperatures as high as 15°C (59°F). *Id.* at page 6, lines 14-17. A person of ordinary skill in the art would understand that collection of a fecal sample at a temperature of 15°C (59°F) corresponds to processing the sample "without freezing." As such, O'Neill teaches the preamble of claim 1.

2) First Limitation of Claim 1

50. The first limitation of claim 1 of the '781 Patent provides "a) collecting a fecal sample from a human subject, wherein the fecal sample is collected at home by the human subject by defecation directly into a sealable collection vessel…". As stated above, a person of ordinary skill in the art would have understood "at home", as used in the '781 Patent, to refer to a location other than the testing facility (e.g., a medical diagnostics laboratory).

51. Lapidus '178 and Lapidus '650 each teach collecting a fecal sample from a human subject at home by defecation directly into a sealable collection vessel. For instance, Lapidus '178 and '650 both describe that "[t]he receptacle may be fitted to a conventional toilet such that the receptacle accepts stool voided in a conventional manner." Ex. 1002 at column 7, lines 64-66; Ex. 1003 at column 7, lines 26-28. This passage provides evidence that the fecal sample is collected from a human subject, since only humans use conventional toilets to void stool in a conventional manner.

52. Furthermore, Lapidus '178 and Lapidus '650 both describe that the fecal sample is collected in a receptacle that can be adapted to fit a toilet, thus demonstrating that the human subject defecates "directly" into the receptacle. As shown in Figure 2 of those references, the receptacle is a "sealable" receptacle comprising an articulating cover 3 that can be utilized in "a closed position (not shown) for sealing voided stool within the receptacle." Ex. 1002 at column 8, lines 24-27; Ex. 1003 at column 7, lines 54-55. Figure 2 is reproduced below:



53. Additionally, Lapidus '178 and Lapidus '650 both describe that the stool can be collected at home. As discussed above, a person of ordinary skill in the art would interpret "at

home," as that term is used in the '781 Patent, to include any location other than a testing facility. Lapidus '178 and Lapidus '650 both describe that the sealable receptacle containing the fecal sample "is preferably small enough to be transported to a testing facility" (Ex. 1002 at column 7, lines 63-64; Ex. 1003 at column 7, lines 24-26), meaning that the fecal sample is collected at *a location different than the testing facility.* Further, Lapidus '178 and Lapidus '650 both describe that the toilet onto which the receptacle may be fitted is "a conventional toilet such that the receptacle accepts stool voided in a conventional manner." Ex. 1002 at column 7, lines 62-64; Ex. 1003 at column 7, lines 26-28.) This indicates to persons skilled in the art that a fecal sample is collected at home by the patient since the "conventional manner" for voiding stool is on a toilet in one's home. In sum, both Lapidus '178 and Lapidus '650 disclose the first limitation of claim 1.

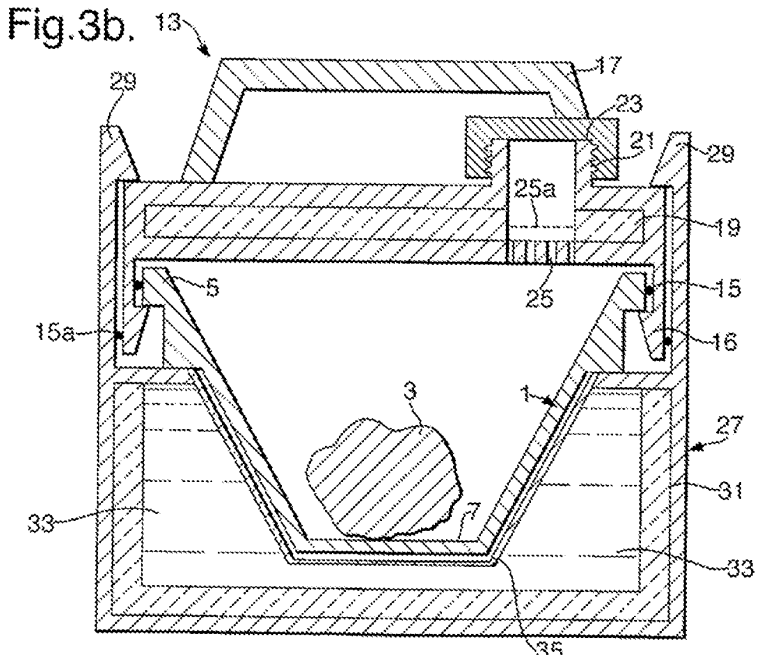
54. Furthermore, any minor differences that may exist between the first limitation of claim 1 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve collecting a fecal sample from a human subject at home by defecation directly into a sealable collection vessel. Thus, at a minimum, this aspect of claim 1 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

55. Ahlquist also teaches collecting a fecal sample from a human subject at home by defecation directly into a sealable collection vessel. Ahlquist discloses that human patients collected stools (a fecal sample) into a plastic bucket mounted to a toilet seat. Ex. 1004 at page 4, at 'Stool Collection and Processing' heading. This indicates to a person skilled in the art that the human subjects in Ahlquist defecated directly into the plastic bucket, which corresponds to the "collection vessel" of claim 1. Further, Ahlquist teaches that the plastic bucket is a sealable collection vessel because the plastic bucket is later sealed upon shipping. *Id.*

56. Additionally, Ahlquist describes that the stool can be collected at home. As discussed above, a person of ordinary skill in the art would interpret "at home," as that term is used in the '781 Patent, to include any location other than a testing facility. Ahlquist discloses that the stools are collected by patients and then express-shipped to the Mayo Clinic, meaning that the fecal sample was collected *at a location different than the Mayo Clinic testing facility.*

This indicates that the stool can be collected in a toilet that is not located at a testing facility. In sum, Ahlquist discloses the first limitation of claim 1.

57. O'Neill also teaches collecting a fecal sample from a human subject at home by defecation directly into a sealable collection vessel. O'Neill discloses that human patients collected stools (a fecal sample) into a container positioned over a toilet. Ex. 1005 at page 8, lines 24-30 ("The container may further have installation means (which may be detachable from the container) for removably installing the container on a toilet (e.g. below the toilet seat) with the receiving mouth opening upwardly and the collection channel extending horizontally beneath the mouth. This allows the user to produce the stool in the usual manner, which promotes user acceptability."). O'Neill teaches that the container is "the receptacle into which the stool is excreted." *Id.* at page 7, line 34- page 8, line 11. Figure 3b of O'Neill is presented below, showing the receptacle (1) of the container that includes an excreted stool (3).



58. O'Neill discloses that "the container is hermetically sealable to reduce the exposure to atmospheric oxygen of a stool deposited therein. The seal may be achieved by means of a lid or cover (e.g. having a snap-fit or screw fitting) for the mouth." *Id.* at page 9, lines 28-33. Figure 3b of O'Neill shows the lid (13) that seals the container of the receptacle (1). Additionally, O'Neill describes that the stool can be collected at home. *Id.* at page 8, lines 7-8

("the stool is excreted into the container at home and then transported to e.g. a clinic for washing"). In sum, O'Neill discloses the first limitation of claim 1.

3) Second Limitation of Claim 1

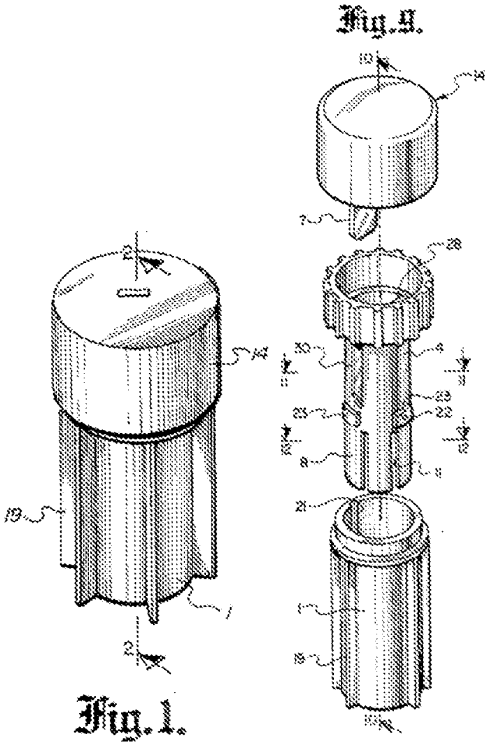
59. The second limitation of claim 1 of the '781 Patent provides "b) removing a portion of the fecal sample to a separate sealable container to produce a removed portion and a remaining portion of the fecal sample …".

60. Lapidus '178 and Lapidus '650 each teach removing a portion of the fecal sample to a separate sealable container, which would produce a 'removed portion' and a 'remaining portion' of the fecal sample. Lapidus '178 and Lapidus '650 both teach that "a cross-sectional portion is removed from the voided stool by making one or more sagittal or coronal section through the stool." Ex. 1002 at column 8, lines 45-49; Ex. 1003 at column 7, lines 17-19. A person of ordinary skill in the art would understand this cross-sectional portion to be a 'removed portion" of the fecal sample recited in claim 1. Lapidus '178 additionally teaches that the 'removed portion' could be in the form of a circumferential surface portion of the voided stool sample or a layer of the voided stool sample that adheres to the surface of a sampling device. Ex. 1002 at column 8, lines 49-52; *id.* at column 9, lines 10-12. In these scenarios, the circumferential surface portion or the layer portion described by Lapidus '178 would correspond to the 'removed portion' of the fecal sample in claim 1. A person of ordinary skill in the art would recognize from the teachings of Lapidus '178 and Lapidus '650 that the portion of the fecal sample left over after removal of a cross-sectional (or a circumferential surface or layer) would be the 'remaining portion' of the fecal sample recited in claim 1.

61. Moreover, Lapidus '178 and Lapidus '650 describe the 'removed portion' of the fecal sample being obtained upon collection of the fecal sample. With reference to Figure 2 (reproduced above), Lapidus '178 and Lapidus '650 each disclose that "[m]eans 8 may be fixedly attached to the support frame 1 or may be removably attached for removal subsequent to deposition of stool. Means 8 may comprise a further means for removing at least a circumferential surface or cross-sectional portion from the voided stool." Ex. 1002 at column 8, lines 33-37; Ex. 1003 at column 7, lines 61-65. The person of ordinary skill in the art would understand this disclosure to teach that the 'removed portion' of the fecal sample (e.g., a circumferential surface or cross-sectional portion from the voided stool) can be obtained in the conventional toilet upon collection of the voided stool.

62. Additionally, Lapidus '178 and Lapidus '650 both teach that the 'removed portion' of the fecal sample is in a separate sealable container. Lapidus '178 and Lapidus '650 describe that the 'removed portion' (e.g., a cross-sectional sample of stool) is placed in a buffer such as phosphate buffered saline according to "means and materials" that are "generally known in the art" in order to assay the 'removed portion'. Ex. 1002 at column 9, lines 30-34; Ex. 1003 at column 8, lines 5-8. At the time we filed the application that led to Lapidus '650 and Lapidus '178, it was well known in the art to use a separate sealable container to homogenize a fecal sample in appropriate buffers for the purpose of assaying. For instance, U.S. Patent No. 4,859,610 to Maggio (Ex. 1010, "Maggio") is one example of the generally known "means and materials" for homogenization being referred to in Lapidus '178 and Lapidus '650.

63. Maggio describes a device that is "particularly useful for homogenizing and analyzing fecal samples." Ex. 1010 at column 4, lines 7-8. Maggio teaches that the device includes a vessel for placement of a fecal sample and that "[a]fter a measured portion of [fecal sample] is secured by the scoop (8) and introduced into the vessel (1), 2 mils of phosphate buffered saline, pH 7.4 (PBS) is added to the vessel (1)." *Id.* at column 8, lines 39-42. Maggio later describes that "FIG. 1 is a perspective view of a preferred embodiment of the immunoassay incubation device." *Id.* at column 3, lines 33-34. Furthermore, Maggio discloses that "FIG. 9 is an exploded view in perspective of another alternative embodiment of the immunoassay incubation device illustrating a valve for controlling the flow of homogenized sample from the extractor into the incubation chamber." *Id.* at column 3, lines 60-64. Figures 1 and 9 of Maggio, reproduced below, clearly depict a separate sealable container that is configured to contain a fecal sample and a homogenizing buffer. Once again, the foregoing disclosure from Maggio exemplifies the type of "homogenization means and materials" being referred to in Lapidus '178 (Ex. 1002 at column 9, lines 30-34) and Lapidus '650 (Ex. 1003 at column 8, lines 5-8), as the person of ordinary skill in the art would have appreciated from the Lapidus disclosure.



64. Furthermore, any minor differences that may exist between the second limitation of claim 1 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve removing a portion of the fecal sample to a separate sealable container such that there would be removed and remaining portions of the sample available for testing. Thus, at a minimum, this aspect of claim 1 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

65. Additionally, De Luca discloses "an extraction and sampling device for collection of faeces samples, particularly for laboratory immunological tests, for qualitative or quantitative determination of one or more analytes, such as haemoglobin…". Ex. 1006 at [0001]. The devices of De Luca are used for detection of blood proteins such as hemoglobin for use in fecal immunochemical blood tests. Further, De Luca teaches that fecal samples are removed and placed in a separate sealable container to perform the fecal immunochemical blood tests. *Id.* at [0015]. The extraction tube of De Luca includes a container body for containing a buffer

solution and fecal sample, and the container is sealed using a cap, rendering the extraction tube a "separate sealable container." *Id.* at [0019], [0032].

66. A person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of Lapidus '178 or Lapidus '650. Specifically, based on the advantages taught in De Luca, the person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Modifying the method of Lapidus '178 or of Lapidus '650 to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 1 based on the combined teachings of Lapidus '178 or Lapidus '650 in view of De Luca.

67. Ahlquist also teaches removing a portion of the fecal sample to produce a 'removed portion' and a 'remaining portion' of the fecal sample. The patients in Ahlquist collected stool and then "smeared stool" onto Hemoccult and HemoccultSensa cards. Ex. 1004 at page 4, at 'Stool Collection and Processing' heading. This smeared stool sample corresponds to the 'removed portion' of the fecal sample in claim 1. The rest of the stool in the plastic bucket disclosed by Ahlquist corresponds to the 'remaining portion' of the fecal sample in claim 1. *Id.*

68. A person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of Ahlquist. 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. *Id.* at page 9, second full paragraph (citations omitted). As discussed above, De Luca teaches the fecal immunochemical blood tests indicated by Ahlquist to provide improved adenoma detection compared to guaiac tests. *See* Ex. 1006 at [0001]. Based on Ahlquist's suggestion to use fecal immunochemical blood tests to "detect more adenomas," as well as the advantages taught in De Luca, a person of ordinary skill in the art would have been motivated to

place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed, as taught in DeLuca. Modifying the method of Ahlquist to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 1 based on the combined teachings of Ahlquist and De Luca.

69. O'Neill also teaches removing a portion of the fecal sample to produce a 'removed portion' and a 'remaining portion' of the fecal sample. O'Neill discloses that cellular material is removed from the fecal sample via a wash liquid and teaches that this 'removed portion' is placed into sample receptacles for subsequent analysis of DNA, RNA and proteins. In particular, O'Neill discloses that "[w]hen the wash programme ends, the sample rack is pivoted about pivot 47 to bring axis 41 into a vertical position (see Fig. 5). Almost all of wash liquid 44 is then decanted out of receptacle 1 via port 21, foraminous screen 25 substantially preventing larger food residues or other broken off pieces from the stool exiting with the liquid. Coarse sieve 25a removes smaller pieces from the liquid. Finer downstream sieves (not shown) further limit the maximum particle size in the liquid. The liquid is then be divided into standard volumes and received into a plurality of removable (e.g. clip-on) sample receptacles (not shown). The sample receptacles may be a strip of tubes each of e.g. 0.1-0.5 ml volume as can be removed and clipped into a standard microplate format used for clinical analyses. Alternatively the sample receptacles may be individual tubes each of e.g. 1-2 ml volume. The sample rack is then returned to its horizontal position and receptacle 1 is removed and safely disposed of." Ex. 1005 at page 14, lines 18-35. A person of ordinary skill in the art would have understood the individual tubes described by O'Neill to be separate, sealable containers, such as Eppendorf tubes that are used in the art. As such, O'Neill teaches "removing a portion of the fecal sample to a separate sealable container." The rest of the stool in the container corresponds to the 'remaining portion' of the fecal sample recited in claim 1.

70. Furthermore, a person of ordinary skill in the art would have found it obvious to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of O'Neill. Based on De Luca's teachings regarding the advantages of such containers, a person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer, as taught in DeLuca. Modifying the method of O'Neill to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 1 based on the combined teachings of O'Neill and De Luca.

4) Third Limitation of Claim 1

71. The third limitation of claim 1 of the '781 Patent provides "c) combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container…".

72. Lapidus '178 and Lapidus '650 each teach combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevents denaturation or degradation of blood proteins found in a fecal sample, and sealing the sealable container. Lapidus '178 and Lapidus '650 both describe combining the removed portion of the fecal sample (e.g., the cross-sectional portion) with a buffer. Ex. 1002 at column 9, lines 30-36; Ex. 1003 at column 8, lines 5-21. Lapidus '178 and Lapidus '650 disclose the buffers as any buffer known to the skilled artisan, such as those that contain detergents and salts, which are well known for preservation of proteins. *Id.* Lapidus '178 and Lapidus '650 further note that to prevent the denaturation or degradation of blood proteins found in the sample, it would be important to specifically avoid including a proteinase in the buffer that would contain detergents and salts. *Id.*

73. As discussed above, with regard to the second limitation of claim 1, Lapidus '178 and Lapidus '650 describe that the cross-sectional portion (and, in Lapidus '178, circumferential surface portion or layer portion) of the fecal sample can be homogenized in a buffer according to "means and materials" that are generally known in the art. Ex. 1002 at column 9, lines 30-34;

Ex. 1003 at column 8, lines 5-8. These generally known "means and materials" included separate sealable containers for combining a removed portion of a sample with a buffer, as evidence by Maggio. *See, e.g.*, Maggio, FIGS. 1, 9; column 3, lines 33-34, 60-64; column 4, lines 7-8; column 8, lines 39-42. Accordingly, Lapidus '178 and Lapidus '650 each taught a person of ordinary skill in the art that the removed portion of the fecal sample and buffer would be combined in a separate sealable container and sealed, according to methods known in the art.

74. Furthermore, any minor differences that may exist between the third limitation of claim 1 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve combining the removed portion of the fecal sample in the separate sealable container with a buffer that prevented denaturation or degradation of blood proteins found in a fecal sample and then sealing the sealable container. Thus, at a minimum, this aspect of claim 1 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

75. Additionally, as discussed above, De Luca discloses placing fecal in a separate sealable containers, with a buffer solution, to perform the fecal immunochemical blood tests. *See, e.g.*, Ex. 1006 at [0001], [0015], [0019], [0032]. As noted above, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of Lapidus '178 or Lapidus '650. Specifically, based on the advantages taught in De Luca, the person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed. Modifying the method of Lapidus '178 or of Lapidus '650 to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the third limitation of claim 1 based on the combined teachings of Lapidus '178 or Lapidus '650 in view of De Luca.

76. Similarly, a person of ordinary skill in the art would have been motivated to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of Ahlquist (i.e., the smeared portions). As discussed above, Ahlquist states that fecal immunochemical blood tests provide improved detection of adenomas compared to guaiac tests, while De Luca teaches such fecal immunochemical blood tests. *See* Ex. 1004 at page 9, second full paragraph; Ex. 1006 at [0001]. Based on Ahlquist's statement suggestion to use fecal immunochemical blood tests to "detect more adenomas," as well as the advantages taught in De Luca, the person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed, as taught in DeLuca. Modifying the method of Ahlquist to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the third limitation of claim 1 based on the combined teachings of Ahlquist and De Luca.

77. Likewise, a person of ordinary skill in the art would also have been motivated to utilize the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of O'Neill (i.e., removed cellular material). O'Neill teaches that the removed portion is placed into sample receptacles for subsequent analysis of DNA, RNA and proteins. Ex. 1005 at page 1, lines 15-20. Thus, a person of ordinary skill in the art reading O'Neill would be motivated to stabilize the removed cellular material so that it could provide a more accurate assessment for patients. Specifically, based on the advantages taught in De Luca, the person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed, as taught in DeLuca. Modifying the method of O'Neill to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of

a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the third limitation of claim 1 based on the combined teachings of O'Neill and De Luca.

5) Fourth Limitation of Claim 1

78. The fourth limitation of claim 1 of the '781 Patent provides "d) combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel."

79. Lapidus '178 and Lapidus '650 each teach combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer, and sealing the sealable collection vessel. As discussed above, with regard to the second limitation of claim 1, a person of ordinary skill in the art would recognize from the teachings of Lapidus '178 and Lapidus '650 that the portion of the fecal sample left over after removal of a cross-sectional (or, in Lapidus '178, a circumferential surface or layer) would be the 'remaining portion' of the fecal sample recited in claim 1. The 'remaining portion' of the fecal sample corresponds to the "stool" in Lapidus '178 and Lapidus '650, which is described as being contained in the receptacle (i.e., the "sealable collection vessel"). Further, Lapidus '178 and Lapidus '650 teach that homogenization buffer can be added to the receptacle containing the stool. Lapidus '178 and Lapidus '650 each state that "[m]ethods of the invention also comprise obtaining a representative (i.e., cross-sectional) ***sample of stool*** and homogenizing ***the stool*** in a buffer, such as a buffer comprising a detergent and a proteinase and optionally a DNase inhibitor. Ex. 1002 at column 3, lines 11-15 (emphasis added); Ex. 1003 at column 2, line 66 – column 3, line 3 (emphasis added). A person of ordinary skill in the art would understand this teaching of Lapidus '178 and Lapidus '650 to refer to two different portions of fecal material – the 'removed portion', or "***sample of stool***", and also the 'remaining portion' of "***the stool***." Lapidus '178 and Lapidus '650 therefore disclose addition of a buffer to the 'remaining portion', i.e., "the stool."

80. Lapidus '178 and Lapidus '650 teach that the buffer can contain phosphate buffered saline, NaCl or KCl, a detergent such as SDS or triton, and inhibitors of DNA and/or RNA degrading enzymes. Ex. 1002 at column 8, lines 5-16; Ex. 1003 at column 7, lines 31-44. A person of ordinary skill in the art would recognize such a buffer as a "stabilizing buffer."

81. Lapidus '178 and Lapidus '650 also disclose that the receptacle includes sealing means and that it should be sealed "to contain the voided stool sample and any solution added thereto and to prevent the emanation of odors." Ex. 1002 at column 8, lines 17-21; Ex. 1003 at column 8, lines 45-49.

82. Furthermore, any minor differences that may exist between the fourth limitation of claim 1 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve combining the remaining portion of the fecal sample in the sealable collection vessel with a stabilizing buffer and then sealing the sealable collection vessel. Thus, at a minimum, this aspect of claim 1 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

83. Additionally, Olson discloses that addition of a stabilizing buffer (Exact buffer A) to stool samples provided a significant increase in recovery of DNA compared with samples incubated without buffer. Olson describes 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 teaches that "[t]he amount of DNA recovered from aliquots stored under the different conditions is based on quantification of a 200-bp DNA fragment (Table 3). Although the amount of recoverable DNA varies widely from sample to sample, at t0 the average recovery was 14,891 copies/10 mL, whereas after room temperature incubation, the average recovery was 1,955. Without buffer added to the samples, between 65% to 98% of the DNA was no longer recoverable (excluding sample GP-031), after incubation of samples at room temperature, compared with t0. The one exception, sample GP-031, maintained high DNA yield even without addition of stabilization buffer. Samples incubated with stabilization buffer maintained human DNA yields similar to the t0 samples (Table 3)." *Id.* at page 186, first column, second full paragraph. In conclusion, Olson states that "DNA can be stabilized by addition of buffer to the sample, leading to increased assay sensitivity. *Id.* at Abstract.

84. A person of ordinary skill in the art would have been motivated to combine the stabilizing buffer of Olson with the 'remaining portion' of the fecal sample of Lapidus '178 or Lapidus '650. Specifically, based on the advantages taught in Olson, the person of ordinary skill

in the art would have been motivated to use the stabilizing buffer taught in Olson to improve DNA assays on the 'remaining portion' of the fecal sample. Modifying the method of Lapidus '178 or of Lapidus '650 to include these teachings of Olson would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the fourth limitation of claim 1 based on the combined teachings of Lapidus '178 or Lapidus '650 in view of Olson.

85. Furthermore, Ahlquist teaches that, after a portion of the fecal sample is removed (by smearing onto the guaiac-based Hemoccult and HemoccultSensa cards), the remaining portion of the fecal sample is "sealed in a bucket" and express-shipped to the testing facility, along with the Hemoccult and HemoccultSensa cards. Ex. 1004 at page 4, at 'Stool Collection and Processing' heading. Although Ahlquist does not teach addition of a stabilizing buffer to the remaining portion of the fecal sample, 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." *Id.* at page 9, third full paragraph (citations omitted). To this end, 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." *Id.* Thus, a person of ordinary skill in the art reading Ahlquist would have been motivated to add a preservative as a stabilizing buffer to the stool at the time of defecation in order to prevent degradation of nucleic acids. As discussed above, Olson teaches such a stabilizing buffer (Exact buffer A). Ex. 1007 at Abstract; page 186, first column, second full paragraph.

86. A person of ordinary skill in the art would have been motivated to combine the stabilizing buffer of Olson with the 'remaining portion' of the fecal sample of Ahlquist, prior to sealing them in the bucket. Specifically, based on the express suggestion in Ahlquist, as well as the advantages taught in Olson, the person of ordinary skill in the art would have been motivated to use the stabilizing buffer taught in Olson to improve DNA assays on the 'remaining portion' of the fecal sample in Ahlquist. Modifying the method of Ahlquist to include these teachings of Olson would have required only minor changes to general methods to stabilize fecal samples

with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the fourth limitation of claim 1 based on the combined teachings of Ahlquist and Olson.

87. Additionally, O'Neill teaches that, after a portion of the fecal sample is removed (by washing to remove cellular material), the remaining portion of the fecal sample is stays in the sealed container. O'Neill states that a typical total stool has a mass of 100-500 grams and that the removed cellular material has a mass of about 50-1000 mg. Ex. 1005 at page 15, lines 1-4. Thus, a person of ordinary skill in the art would understand that O'Neill teaches a remaining portion of the stool of at least 99 grams that remains in the container. Although O'Neill does not teach addition of a stabilizing buffer to the remaining portion of the fecal sample, a person of ordinary skill in the art would have been motivated to stabilize the remaining stool sample so that subsequent analyses could be made and/or improved. As discussed above, Olson teaches such a stabilizing buffer (Exact buffer A). Ex. 1007 at Abstract; page 186, first column, second full paragraph.

88. A person of ordinary skill in the art would have been motivated to combine the stabilizing buffer of Olson with the 'remaining portion' of the fecal sample of O'Neill. Specifically, based on the advantages taught in Olson, the person of ordinary skill in the art would have been motivated to use the stabilizing buffer taught in Olson to improve DNA assays on the 'remaining portion' of the fecal sample in O'Neill. Modifying the method of O'Neill to include these teachings of Olson would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the fourth limitation of claim 1 based on the combined teachings of O'Neill and Olson.

6) Conclusions Regarding Claim 1

89. For the reasons discussed above, it is my opinion that Lapidus '178 described each and every element of claim 1 arranged in the same way as in that claim. Therefore, Lapidus '178 anticipated claim 1.

90. For the reasons discussed above, it is also my opinion that Lapidus '178 suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing. Therefore, Lapidus '178 rendered claim 1 obvious.

91. For the reasons discussed above, it is also my opinion that Lapidus '650 described each and every element of claim 1 arranged in the same way as in that claim. Therefore, Lapidus '650 anticipated claim 1.

92. For the reasons discussed above, it is also my opinion that Lapidus '650 suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing. Therefore, Lapidus '650 rendered claim 1 obvious.

93. For the reasons discussed above, it is also my opinion that Lapidus '178, De Luca, and Olson collectively taught or suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 1. Therefore, Lapidus '178, De Luca, and Olson rendered claim 1 obvious.

94. For the reasons discussed above, it is also my opinion that Lapidus '650, De Luca, and Olson collectively taught or suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 1. Therefore, Lapidus '650, De Luca, and Olson rendered claim 1 obvious.

95. For the reasons discussed above, it is also my opinion that Ahlquist, De Luca, and Olson collectively taught or suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 1. Therefore, Ahlquist, De Luca, and Olson rendered claim 1 obvious.

96. For the reasons discussed above, it is also my opinion that O'Neill, De Luca, and Olson collectively taught or suggested each and every element of claim 1 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those

teachings to arrive at the subject matter of claim 1. Therefore, O'Neill, De Luca, and Olson rendered claim 1 obvious.

B. Claim 2 Was Anticipated and Obvious

97. Claim 2 of the '781 Patent provides "[t]he method of claim 1, further comprising delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory."

98. Lapidus '178 and Lapidus '650 each teach "that "[s]tool is voided into a receptacle that is preferably small enough to be transported to a testing facility." Ex. 1002 at column 7, lines 63-64; Ex. 1003 at column 7, lines 24-26. A person of ordinary skill in the art would understand that 'a testing facility' as described in Lapidus '178 and Lapidus '650 would correspond to 'a medical diagnostics laboratory' according to the usual meaning in the field. Thus, both Lapidus '178 and Lapidus '650 disclose claim 2.

99. Furthermore, any minor differences that may exist between claim 2 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve delivering the sealable container containing the removed portion of the fecal sample and said buffer and the sealable collection vessel containing the remaining portion of the fecal sample and said stabilizing buffer to a medical diagnostics laboratory. Thus, at a minimum, claim 2 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

100. Furthermore, 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. A person of ordinary skill in the art would understand that the Mayo Clinic as taught by Ahlquist would indicate 'a medical diagnostics laboratory' according to the usual meaning in the field. Thus, Ahlquist teaches claim 2.

101. Moreover, O'Neill teaches 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." *Id.* at page 8, lines 7-11. A person of ordinary skill in the art would understand that the clinic as taught by O'Neill would indicate 'a medical diagnostics laboratory' according to the usual meaning in the field. Thus, O'Neill teaches claim 2.

102. As taught by Lapidus '178, Lapidus '650, Ahlquist, and O'Neill, it was routine in the art for the method of processing to include collection of fecal samples at a place other than a medical diagnostics laboratory and then delivery of the fecal samples to the medical diagnostics laboratory. For instance, a person of ordinary skill in the art would be motivated to utilize the teachings of Lapidus '178, Lapidus '650, Ahlquist, and/or O'Neill to collect fecal samples at one location and then deliver them to a medical diagnostics laboratory since each reference suggests that these steps could be undertaken. All such teachings were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results.

103. In summary, for the reasons discussed above, it is my opinion that Lapidus '178 described each and every element of claim 2. Therefore, Lapidus '178 anticipated claim 2.

104. For the reasons discussed above, it is also my opinion that Lapidus '178 suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing. Therefore, Lapidus '178 rendered claim 2 obvious.

105. For the reasons discussed above, it is also my opinion that Lapidus '650 described each and every element of claim 2. Therefore, Lapidus '650 anticipated claim 2.

106. For the reasons discussed above, it is also my opinion that Lapidus '650 suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing. Therefore, Lapidus '650 rendered claim 2 obvious.

107. For the reasons discussed above, it is also my opinion that Lapidus '178, De Luca, and Olson collectively taught or suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 2. Therefore, Lapidus '178, De Luca, and Olson rendered claim 2 obvious.

108. For the reasons discussed above, it is also my opinion that Lapidus '650, De Luca, and Olson collectively taught or suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 2. Therefore, Lapidus '650, De Luca, and Olson rendered claim 2 obvious.

109. For the reasons discussed above, it is also my opinion that Ahlquist, De Luca, and Olson collectively taught or suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 2. Therefore, Ahlquist, De Luca, and Olson rendered claim 2 obvious.

110. For the reasons discussed above, it is also my opinion that O'Neill, De Luca, and Olson collectively taught or suggested each and every element of claim 2 to those skilled in the art prior to the '781 Patent's filing and that it would have been obvious to combine those teachings to arrive at the subject matter of claim 2. Therefore, O'Neill, De Luca, and Olson rendered claim 2 obvious.

C. Claim 3 Was Anticipated and Obvious

1) Preamble of Claim 3

111. The preamble of claim 3 of the '781 Patent provides "[a] method of processing a fecal sample, the method comprising …". As discussed above in relation to claim 1, a person of ordinary skill in the art would have understood that each of Lapidus '178, Lapidus '650, Ahlquist, and O'Neill teach methods of processing a fecal sample. As such, each of Lapidus '178, Lapidus '650, Ahlquist, and O'Neill teach the preamble of claim 3.

2) First Limitation of Claim 3

112. The first limitation of claim 3 of the '781 Patent provides "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 …". As discussed above in relation to claim 1, a person of ordinary skill in the art would have understood that each of Lapidus '178, Lapidus '650, Ahlquist, and O'Neill taught and/or suggested obtaining a pair of

portions of a fecal sample collected from a human subject that are obtained by the method of claim 1.

113. Accordingly, both Lapidus '178 and Lapidus '650 disclose the first limitation of claim 3. Furthermore, any minor differences that may exist between the first limitation of claim 3 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Moreover, Ahlquist discloses the first limitation of claim 3. In addition, O'Neill discloses the first limitation of claim 3.

3) Second Limitation of Claim 3

114. The second limitation of claim 3 of the '781 Patent provides "b) testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion …".

115. Lapidus '178 and Lapidus '650 each testing the removed portion of the fecal sample for an amount of a blood protein present in the removed portion. Lapidus '178 and Lapidus '650 both teach that after sample (e.g., a 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; Ex. 1003 at column 6, lines 1-11. Lapidus '178 and Lapidus '650 additionally teach 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." Ex. 1002 at column 9, lines 40-45; Ex. 1003 at column 8, lines 13-18.

116. Furthermore, any minor differences that may exist between the second limitation of claim 3 and the teachings of Lapidus '178 and Lapidus '650 would have been obvious to a person of ordinary skill in the art at the time of the invention. Indeed, at the time we obtained the Lapidus '178 and Lapidus '650 patents, I envisioned that the methods described in those references could and would involve testing the removed portion of the fecal sample for an

amount of a blood protein present in the removed portion. Thus, at a minimum, this aspect of claim 3 was obvious in view of the teachings of Lapidus '178 and/or Lapidus '650.

117. In addition, 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. *Id.* 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." *Id.* at [0048].

118. A person of ordinary skill in the art would have been motivated to utilize the laboratory immunological tests of De Luca to analyze hemoglobin in the fecal sample of Lapidus '178 and/or Lapidus '650. Lapidus '178 and Lapidus '650 disclose that testing blood proteins was a goal of their analyses. As discussed above, De Luca teaches fecal immunochemical blood tests that can improve improved adenoma detection. *See* Ex. 1006 at [0001]. These advantages taught in De Luca would have motivated a person of ordinary skill in the art to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test could be performed, as taught in DeLuca. Modifying the method of Lapidus '178 or Lapidus '650 to include these teachings of De Luca would have required only minor changes to general methods to test fecal samples for hemoglobin, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 3 based on the combined teachings of Lapidus '178 or Lapidus '650 and De Luca.

119. Ahlquist teaches that "[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.

120. A person of ordinary skill in the art would have been motivated to analyze hemoglobin in the fecal sample of Ahlquist. 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. *Id.* at page 9, second full paragraph (citations omitted). As discussed above, De Luca teaches the fecal immunochemical blood tests indicated by Ahlquist to provide improved adenoma detection compared to guaiac tests by testing for a blood protein such as hemoglobin. *See* Ex. 1006 at [0001]. Based on Ahlquist's suggestion to use fecal immunochemical blood tests to "detect more adenomas," as well as the advantages taught in De Luca, a person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer in the extraction tube's container body to prevent denaturation or degradation of blood proteins so that a fecal immunochemical blood test on blood proteins could be performed, as taught in DeLuca. Modifying the method of Ahlquist to include these teachings of De Luca would have required only minor changes to general methods to stabilize fecal samples with buffer in sealable containers, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 3 based on the combined teachings of Ahlquist and De Luca.

121. O'Neill teaches "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." *Id.* at page 1, lines 15-24.

122. Furthermore, a person of ordinary skill in the art would have found it obvious to test for a blood protein such as hemoglobin via the separate sealable container of De Luca to receive the 'removed portion' of the fecal sample of O'Neill. Based on De Luca's teachings regarding the advantages of such containers, a person of ordinary skill in the art would have been motivated to place the removed portion of the fecal sample with a buffer, as taught in DeLuca, and test for a blood protein such as hemoglobin. Modifying the method of O'Neill to include these teachings of De Luca would have required only minor changes to general methods to test for a blood protein such as hemoglobin, all of which were well within the knowledge and technical grasp of a person of ordinary skill in the art, who would therefore have a reasonable expectation of success. It also would have been a predictable use of the prior art according to their established functions and yielded predictable results. As such, one skilled in the art would have found it obvious to implement the second limitation of claim 3 based on the combined teachings of O'Neill and De Luca.

4) Third Limitation of Claim 3

123. The third limitation of claim 3 of the '781 Patent provides "c) extracting nucleic acid from the remaining portion of the fecal sample …".

124. Lapidus '178 and Lapidus '650 each teach extracting nucleic acid from the remaining portion of the fecal sample. Lapidus '178 and Lapidus '650 both teach diagnostic assays can be performed on a whole stool or a portion that "contains sufficient material to allow subsequent diagnostic assays to be performed." Ex. 1002 at column 9, lines 26-29; Ex. 1003 at column 9, lines 21-24. Lapidus '178 and Lapidus '650 teach 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 U.S. Pat. No. 5,670,325 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. 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