SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Blood-based Qualitative Colorectal Cancer Screening Test

Device Trade Name: Epi proColon®

Device Procode: PHP

Applicant's Name and Address: Epigenomics AG
Genesteastr. 5
10829 Berlin
Germany

Date of Panel Recommendation: March 26, 2014

Premarket Approval Application (PMA) Number: P130001

Date of FDA Notice of Approval: April 12, 2016

Priority Review: Granted priority review status on February 15, 2013, because Epi proColon® is a first of a kind device that uses breakthrough technology.

II. INDICATIONS FOR USE

The Epi proColon test is a qualitative *in vitro* diagnostic test for the detection of methylated Septin 9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the SEPT9_v2 transcript has been associated with the occurrence of colorectal cancer (CRC). The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin 9 DNA target.

The Epi proColon test is indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC, who have been offered and have a history of not completing CRC screening. Tests that are available and recommended in the USPSTF 2008 CRC screening guidelines should be offered and declined prior to offering the Epi proColon test. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. The Epi proColon test results should be used in combination with physician's assessment and individual risk factors in guiding patient management.

III. CONTRAINDICATIONS

The Epi proColon test is not intended to replace CRC screening tests that are recommended by appropriate guidelines (e.g., 2008 USPSTF guidelines) such as colonoscopy, sigmoidoscopy and high sensitivity fecal occult blood testing.

The Epi proColon test is not intended for patients who are willing and able to undergo routine CRC screening tests that are recommended by appropriate guidelines.

The Epi proColon test is not intended for patients defined as having elevated risk for developing CRC based on previous history of colorectal polyps, CRC or related cancers, inflammatory bowel disease (IBD), chronic ulcerative colitis (CUC), Crohn's disease, familial adenomatous polyposis (FAP). Persons at higher risk also include those with a family history of CRC, particularly with two or more first degree relatives with CRC, or one or more first degree relative(s) less than 50 years of age with CRC.

The Epi proColon test has not been evaluated in patients who have been diagnosed with a relevant familial (hereditary) cancer syndrome, such as non-polyposis CRC (HNPCC or Lynch Syndrome), Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner's syndrome, Turcot's (or Crail's) syndrome, Cowden's syndrome, Juvenile Polyposis, Cronkhite-Canada syndrome, Neurofibromatosis, or Familial Hyperplastic Polyposis, or in patients with anorectal bleeding, hematochezia, or with known iron deficiency anemia.

IV. WARNINGS AND PRECAUTIONS

The Epi proColon test demonstrated inferiority to a fecal test (OC FIT-CHEK® Polymedco, Inc.) for specificity, indicating that the Epi proColon test exhibited a higher rate of false positive results compared to the FIT test. The Epi proColon demonstrated non-inferiority to a fecal test for sensitivity.

A positive Epi proColon test result is not confirmatory evidence for CRC. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy.

A negative Epi proColon test result does not guarantee absence of cancer. Patients with a negative Epi proColon test result should be advised to continue participating in a recommended CRC screening program according to screening guidelines.

Screening with Epi proColon in subsequent years following a negative test result should be offered only to patients who after counseling by their healthcare provider, again decline CRC screening methods according to appropriate guidelines. The screening interval for this follow-up has not been established.

The performance of Epi proColon has been established in cross-sectional (i.e., single point in time) studies. Programmatic performance of Epi proColon (i.e., benefits and risks with repeated testing over an established period of time) has not been studied. Performance has not been evaluated for patients who have been previously tested with Epi proColon. Non-inferiority of Epi proColon programmatic sensitivity as compared to other recommended screening methods for CRC has not been established.

The rate of false positive Epi proColon results increases with age. Test results should be interpreted with caution in elderly patients.

CRC screening guideline recommendations vary for persons over the age of 75. The decision to screen persons over the age of 75 should be made on an individualized basis in consultation with a healthcare provider.

Positive test results have been observed in healthy subjects and in patients diagnosed with chronic gastritis, lung cancer, and in pregnant women.

Test results should be interpreted by a healthcare professional. Patients should be advised of the cautions listed in the Epi proColon Patient Guide.

V. DEVICE DESCRIPTION

The Epi proColon test is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of methylated Septin 9 DNA isolated from 3.5 mL of patient plasma. Frequently, there is increased methylation of cytosine residues in the v2 region of the Septin 9 gene in CRC tissue. The detection of methylated Septin 9 DNA in plasma is associated with the occurrence of CRC.

The Epi proColon test includes three components:

1) The Epi proColon Plasma Quick Kit
2) The Epi proColon Sensitive PCR Kit
3) The Epi proColon Control Kit

Epi proColon Plasma Quick Kit

The kit is shipped at ambient temperature and stored at room temperature (15-30°C). The kit contents are sufficient to process 32 samples. The contents of the kit are listed in Table 1.

Table 1. Epi proColon Plasma Quick Kit Contents

Reagent	Volume
Epi proColon Lysis Binding Buffer	125 mL x 1 bottle
Epi proColon Magnetic Beads	4 mL x 1 bottle
Epi proColon Wash A Concentrate	60 mL x 1 bottle
Epi proColon Elution Buffer	6 mL x 1 tube
Epi proColon Bisulfite Solution	1.9 mL x 4 tubes
Epi proColon Protection Buffer	1 mL x 1 tube
Epi proColon Wash B Concentrate	7 mL x 1 bottle

Epi proColon Sensitive PCR Kit

The kit is shipped frozen and stored at -25 to -15°C. The kit contents are sufficient to test 32 samples. The contents of the kit are listed in Table 2.

Table 2. Epi proColon Sensitive PCR Kit Contents

Reagent	Volume
Epi proColon PCR Mix	810 µL x 2 tubes
Epi proColon Polymerase	85 µL x 1 tube

Epi proColon Control Kit

The kit is shipped frozen and stored at -25 to -15°C. The kit contents are sufficient for 6 independent runs, and are listed in Table 3.

Table 3. Epi proColon Control Kit Contents

Reagent	Volume
Epi proColon Positive Control	3.65 mL x 6 tubes
Epi proColon Negative Control	3.65 mL x 6 tubes

Materials Required, But Not Provided

Blood Collection Tube
The Epi proColon test is intended for use with BD Vacutainer® K2 EDTA blood collection tubes.

Real-Time PCR Instrument and Software
The Epi proColon test is intended for use with the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument using the Sequence Detection Software v1.4. No additional software is required to analyze or interpret the test results.

Additional materials required, but not provided by Epigenomics, are listed in the Epi proColon Instructions for Use.

Principles of Operation

The Epi proColon test is predicated on the observation that cancer specific DNA can be detected in the blood of individuals with colorectal cancer [1]. It is thought that DNA from tumor cells can enter the blood stream through necrosis or apoptosis of the malignant cells. The Epi proColon test identifies a region of the v2 promoter of the Septin 9 gene that has increased methylation in CRC tissue and has been detected in plasma of individuals with CRC [2].

Approximately 10 mL blood is collected in BD Vacutainer® K2 EDTA tubes. Plasma is prepared within 4 hours after the blood draw, according to Epi proColon instructions for use (IFU). Plasma is separated from whole blood by centrifugation and then transferred to a new tube. The spin and transfer process is repeated for a second time. The plasma may be used immediately or stored at -25°C to -15°C.

The Epi proColon Plasma Quick Kit is used for the extraction, purification and conversion of DNA from plasma. DNA is extracted from 3.5 mL plasma using magnetic beads. After wash and elution steps, the isolated DNA is treated with bisulfite solution. The bisulfite treatment converts unmethylated cytosines to uracils, while leaving

methylated cytosines (5-methylcytosine) unaffected. This allows for differentiation of methylated and unmethylated cytosines. The bisulfite-treated DNA is then isolated with the magnetic beads, washed, and eluted.

The Epi proColon Sensitive PCR Kit is used to amplify and detect the methylated Septin 9 (mSEPT9) target region and a control region in the β-actin gene (ACTB) in one PCR reaction. The bisulfite-treated DNA is aliquotted to the PCR reaction wells, along with the PCR mix and polymerase, such that each sample is run in triplicate. Real-time PCR is performed on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument using the Sequence Detection Software v1.4. The assay is designed to preferentially amplify the mSEPT9 target region by using a combination of a blocker oligonucleotide and a methylation-specific fluorescent probe. The blocker oligonucleotide binds to unmethylated bisulfite-treated sequence in the target region and thereby prevents amplification of unmethylated sequences. The methylated target sequence, which is amplified, is detected by a fluorescently labeled methylation-specific probe.

The validity of each run is determined by the run controls, which are provided in the Epi proColon Control Kit. Both the positive and negative controls must meet the specified cutoff criteria for the run to be valid and for sample results to be analyzed. Each replicate is assessed relative to specified cycle threshold (Ct) values for ACTB and mSEPT9. A 'positive' result is reported if at least one PCR reaction (out of three replicates) yields ACTB and mSEPT9 Ct values that do not exceed the specified thresholds. If all sample replicates have valid ACTB Ct values and undetermined mSEPT9 Ct values, then a 'negative' result is reported. If the criteria for neither a positive nor negative result are met, then the result is invalid. A qualitative result is reported.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

The CRC screening tests that are recommended by most, if not all, professional societies include both invasive tests (colonoscopy, flexible sigmoidoscopy) and non-invasive fecal occult blood tests (including both guaiac fecal occult blood tests and fecal immunochemical tests). Each alternative has its own advantages and disadvantages. Colonoscopy is considered the most accurate screening method available, which can involve the removal of precancerous lesions to prevent cancer. Non-invasive tests can be preferred in some cases over invasive tests based on cost or patient preference. A patient should fully discuss these alternatives with his/her health care provider to select the method that is most appropriate. Patients who have a positive test by an invasive or non-invasive screening method, with the exception of colonscopy itself, warrant further investigation through conventional colonoscopy.

VII. MARKETING HISTORY

Epi proColon has been marketed as Epi proColon® 2.0 CE since 2011 in the European Union and the Asian Pacific region. The test replaced the first generation test that was first distributed commercially as a CE-marked test in Europe and the Middle East in

2009. This test has not been withdrawn from marketing for any reasons due to safety and effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The Epi proColon test measures DNA in plasma, and therefore requires a minimally invasive blood draw. The risk for potential adverse effects associated with this procedure is minimal.

The primary risk associated with the Epi proColon test is the occurrence of a false negative result. A false negative result would delay follow-up diagnostic procedures, such as colonoscopy. There is also a risk of false positive results, which may lead to patients being referred to colonoscopy unnecessarily. As a result, patients may experience adverse events related to the colonoscopy procedure.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

i. Cutoff Determination

The cutoffs and validity limits were determined for the Epi proColon test based on an evaluation of a panel of donor samples that were categorized by colonoscopy. Blood was collected and processed to plasma from a total of 103 subjects with CRC and 100 subjects with no evidence of disease (NED). CRC subjects are defined as those with invasive CRC, stages I-IV, and NED includes those with no evidence of CRC, polyps or other gastrointestinal diseases, as determined by colonoscopy. Run controls were included in each run. The results are shown in the table below. The parameter settings were selected based on test positivity in CRC cases, and the cycle threshold values for mSEPT9 and ACTB were confirmed in this study.

Table 4. Test Results for Clinically Defined Categories

Specimen	Number Tested	Valid Result	mSEPT9 Positive
NED	100	99	16
CRC	103	98	93
Stage I	29	27	24
Stage II	32	29	27
Stage III	31	31	31
Stage IV	11	11	11
Total	203	197	109

ii. Analytical Sensitivity – Limit of Detection

The limit of detection (LoD) of Epi proColon was determined following CLSI guidelines EP12-A2 (Evaluation of Qualitative Test Performance) and EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures). Six concentration levels (ranging from 1.5 to 50 pg/mL) of technical samples spiked with HeLa cell line DNA were tested with multiple kit lots. A minimum of 60 replicates were tested. Samples consisting of cell line DNA spiked into human plasma at the same levels were also evaluated. The LoD based on the plasma samples was 4.7 pg/mL (95% CI: 2.5 – 9.0 pg/mL). The LoD of the assay was determined to be above the assay cutoff.

Samples without DNA (0 pg/mL) were also evaluated and did not result in a Septin 9 signal.

iii. <u>Analytical Specificity</u>

a) Primer and Probe Specificity

The assay is designed to specifically amplify and detect a methylated region in the Septin 9 gene (mSEPT9). Alignment analyses using Ensembl Genome Browser-Blast and the NCBI database were performed for the amplicon regions for mSEPT9 and ACTB. For mSEPT9, one single nucleotide polymorphism (SNP) was detected, which should not affect the amplification of the expected product due to the resulting sequence after bisulfite conversion. For ACTB, three SNPs were identified, and are not expected to influence the performance of the assay due to the low frequency of the alleles or due to the resulting sequence after bisulfite conversion.

In silico PCR was performed using the NCBI database for the mSEPT9 and ACTB target regions. In both cases, no amplicon other than the expected amplicons was detected using the least restrictive conditions, which allowed for a 200-base pair (bp) size deviation, a 2 bp mismatch per primer, and a 2 bp gap in each primer.

b) Cross Reactivity

Specificity for the fully methylated mSEPT9 target sequence was empirically demonstrated using synthetic templates representing all possible combinations of methylation at the CpG sites with the mSEPT9 probe. Real-time PCR followed by melting curve analysis was used. Fluorescence was not detected with the partially methylated templates. A fluorescence signal was exclusively detected with the fully methylated sequence, indicating that a positive test result requires complete methylation at the CpG sites in the mSEPT9 probe.

c) Methylation Testing

The specificity of DNA methylation of Epi proColon was tested against methylated Septin 9 DNA targets as determined by Sanger sequencing. In one study, nine bulk plasma samples were spiked with HeLa cell DNA (methylated at Septin 9). The PCR products were subcloned and 24 clones

per sample were sequenced. A total of 199 conclusive sequences were analyzed. Among 595 CpG sites, 585 were methylated and out of 476 potential convertible cytosines, 473 were converted. No mSEPT9 sequences were obtained from the negative control sample. In another study, PCR products of 10 positive non-CRC samples (with positive Epi proColon results), and 3 positive control samples were subcloned and sequenced. For all samples, 24 subclones were picked and sequenced. Out of 312 sequences, 217 were readable. The overall number of unconverted cytosines was below 0.5%, indicating a high conversion rate. Sequencing confirmed that mSEPT9 signals detected by Epi proColon are consistent with the presence of methylated SEPT9 sequence in all tested control and patient samples.

d) Cross-Reactivity with Non-Tumor Chronic Conditions

The performance of Epi proColon was evaluated in subjects with non-cancer diseases. Some patients were reported to have multiple conditions and were, therefore, counted in each relevant category. Among 193 patients with chronic diseases, 191 had valid test results and 33 of those tested positive for mSEPT9. Aside from the small number effects, none of the categories had positive detection fractions significantly different from the overall proportion of positive test results (χ^2 test p-value = 0.54). Most categories with less than 10 specimens had positivity rates greater than those observed for the non-CRC groups in the clinical studies (Table 5).

Table 5. Positive Detection Fraction (PDF) by Condition in Non-Tumor Subjects

Co-morbidity	Total	Valid	Neg	Pos	PDF (%)	95% CI
Arterial hypertension	104	103	85	18	17	11, 26
Cardiovascular disease	17	17	14	3	18	6, 41
Chronic gastritis	17	17	12	5	29	13, 53
Chronic obstructive pulmonary disease	10	10	9	1	10	1, 40
Diverticulosis	1	1	0	1	100	5, 100
Diverticulitis	3	3	2	1	33	2, 79
Esophagitis	8	8	6	2	25	7, 59
Hyperlipidemia	34	34	29	5	15	6, 30
Inflammatory bowel disease	7	7	6	1	14	1, 51
Nephritis/nephrosis	2	2	2	0	0	0, 66
Other chronic disease	56	55	46	9	16	9, 28
Other liver disease	3	3	2	1	33	2, 79
Rheumatoid arthritis (RA)	4	4	4	0	0	0, 49
Non-RA	10	10	9	1	10	1, 40
Other rheumatic condition	8	7	7	0	0	0, 35
Type II diabetes	22	21	20	1	5	0, 23
None	10	10	9	1	10	1, 40

e) Cross-Reactivity with Non-Colorectal Cancers

The performance of Epi proColon was evaluated in subjects with non-CRC related cancers. There were limited numbers of subjects in each cancer category, except for breast, lung, and prostate cancers (Table 6). The positivity rate in patients with lung cancer is substantially larger than that observed for the non-CRC groups in clinical Study 1 (p-value < 0.001) and Study 2 (p-values < 0.001).

Table 6. Positive Detection Fraction (PDF) by Tumor Category

Cancer	Total	Valid	Neg	Pos	PDF (%)	95% CI
CRC	22	22	3	19	86	67, 95
Bladder	4	3	2	1	33	2, 79
Breast	23	22	18	4	18	7, 39
Hepatocellular Carcinoma	1	1	0	1	100	5, 100
Kidney	3	3	1	2	67	21, 98
Lung	102	99	46	53	54	43, 64
Prostate	41	40	30	10	25	14, 40
Skin melanoma	1	1	1	0	0	0, 95
Stomach	1	1	0	1	100	5, 100
Other	3	3	3	0	0	0, 56

f) Interference

The effects of 10 potential interfering substances on the performance of Epi proColon were evaluated (Table 7). Three batches of 22 samples were processed using one lot of reagents, resulting in the analysis of 3 runs per sample per interfering substance. The samples used for testing were composed of human bulk plasma, such that 10 plasma samples contained interfering substance (i.e., one for each substance), 10 plasma samples contained interfering substance and HeLa cell DNA (mDNA) at 35 pg/mL, 1 plasma sample did not contain interfering substance, and 1 plasma sample without interfering substance was spiked with HeLa cell DNA at 35 pg/mL. False positive results were detected when 3 substances were tested at higher concentrations: albumin (40 mg/mL), red blood cells (0.4% v/v) and human sperm DNA (100 ng/mL).

Table 7. Results for Interfering Substances

Interfering substance	Concentration	mDNA	Neg	Pos
Bilirubin	0.2 mg/mL	Yes No	0 3	3 0
Cholesterol	5 mg/mL	Yes No	0 3	3 0
Glucose	10 mg/mL	Yes No	0 3	3 0
Hemoglobin	10 mg/mL	Yes No	0 3	3 0
Protein (albumin)	40 mg/mL	Yes No	0 2	3 1
Triglycerides	12 mg/mL	Yes No	0 3	3 0
Uric acid	0.235 mg/mL	Yes No	0 3	3 0
K_2EDTA	20 mg/mL	Yes No	0 3	3 0
RBCs	0.4% v/v	Yes No	0 2	3 1
Unmethylated gDNA (human sperm)	100 ng/mL	Yes No	0 2	3 1
None	--	Yes No	0 3	3 0

iv. <u>Precision and Reproducibility</u>

Two studies were conducted to assess reproducibility of the Epi proColon assay. One study was performed to validate that the test generates consistent results when different lots, runs and operators are used. Repeatability (% agreement with clinical diagnosis) of test results was evaluated at one site by testing six batches of 9 samples (6 CRC sample pools and 3 pools from self-declared healthy blood donors); one batch was included per run. One positive control and one negative control were included as batch controls. The study included three operators, each of whom used a different reagent lot. Acceptance criterion was that the observed agreement is $\geq$ 90%. All batches were valid, and the positive agreement rate was 100% for CRC samples. The agreement rate for healthy subject pools with negative test results was 88.9% (16/18). Taken together, 96% (52/54) results agreed.

Another study was performed to estimate the consistency of test results when clinical material was assayed at various sites by different operators using different reagent lots. This study assessed site-to-site, lot-to-lot, operator-to-operator, run-to-run, and day-to-day variability. A total of 14 clinical sample pools were tested

at three sites with six operators (two per site) using three reagent lots and three PCR instruments. Identical sets of samples were sent to each site. Six pools (Pools 1-6) were generated from CRC plasma, three pools (Pools 7-9) were from self-declared healthy blood donors, and 5 pools (Pools 10-14) were prepared by diluting a CRC plasma aliquot into human bulk plasma. Across all sites, each pool was assayed 12 times. The reproducibility results for mSEPT9 and ACTB are listed in Tables 8 and 9, respectively.

For reproducibility, the standard deviation ranges for mSEPT9 are 0.4 to 2.3 Ct. The corresponding ranges for ACTB are 0.2 to 0.4 Ct. The expected test result of a CRC sample is a positive result, and the expected test result of a non-CRC sample is a negative result. The agreement with the expected test result in replicate testing for all CRC pools was 98% (95% CI: 94% – 99%). The agreement with the expected test result in replicate testing for the healthy donor pools was 75% (95% CI: 59% – 86%).

Table 8. Reproducibility Results for mSEPT9

Pool	Mean Ct	Variance Component - SD						
		Batch	Day*	Operator	Kit	Site	Residual	Total
1	36.96	0.47	-	0.11	0	0.95	0.94	1.42
2	36.39	0.23	-	0.36	0.48	0.57	1.00	1.32
3	34.16	0	-	0.28	0.17	0.62	0.45	0.83
4	29.81	0.27	-	0	0	0.19	0.23	0.40
5	36.14	0.11	-	0	0.95	0.54	0.90	1.42
6	30.37	0.26	-	0	0	0.42	0.22	0.54
10	38.85	0	0.81	0	0	1.48	1.05	1.99
11	39.52	0	0.45	0	0	0	1.52	1.58
12	36.89	0	0.79	0	0	0	1.48	1.68
13	38.65	0	0	0	0	0.95	2.11	2.31
14	38.59	0	0.52	0	0	1.01	1.00	1.51

* Due to the nested study design, between-day variation cannot be differentiated from between-batch variation for pools 1-9.

Table 9. Reproducibility Results for ACTB

Pool	Mean Ct	Variance Component - SD						
		Batch	Day*	Operator	Kit	Site	Residual	Total
1	27.54	0.17	-	0	0	0	0.11	0.20
2	26.13	0.22	-	0	0	0	0.07	0.23
3	27.59	0.21	-	0	0.08	0.09	0.10	0.26
4	27.20	0.22	-	0	0	0	0.13	0.25
5	27.32	0.05	-	0.06	0.21	0	0.17	0.28
6	27.47	0.23	-	0	0.08	0	0.14	0.28
7	27.14	0.17	-	0	0.08	0	0.19	0.27
8	27.79	0.28	-	0	0	0.20	0.09	0.35
9	28.13	0.18	-	0	0	0	0.10	0.20
10	26.76	0.10	0	0.16	0	0	0.28	0.31
11	23.22	0	0	0.23	0.11	0	0.27	0.37
12	26.99	0	0.16	0.17	0	0	0.12	0.27
13	26.61	0.04	0	0.20	0	0	0.15	0.25
14	25.07	0	0.26	0	0	0	0.10	0.28

* Due to the nested study design, between-day variation cannot be differentiated from between-batch variation for pools 1-9.

v. <u>Robustness</u>

a) Failure Modes

Twenty potential failure modes were tested. Ten modes were related to DNA extraction, 2 modes were related to bisulfite conversion reaction, 3 modes were related to DNA purification, and 5 modes were related to PCR amplification. The study was carried out by two operators using 5 sample batches. Negative plasma specimens from human bulk plasma and plasma specimens spiked with 35 pg/mL HeLa cell DNA were tested. In addition, 1 positive control and 1 negative control were included. All tested failure modes were considered uncritical (i.e., produced a valid test result) or identified critical failure mode (i.e., produced invalid test results).

b) Bisulfite Conversion

The robustness of the bisulfite conversion was also tested. One negative plasma sample, one analyte positive plasma samples, 1 positive control and 1 negative control were evaluated. Parameters that could potentially result in incomplete conversion were evaluated: reaction time (5-45 min) and temperature (30-95°C). Some conditions had no effect on test results, while others were identified as a failure mode due to invalid external and/or internal controls. There were no false positive or false negative results, suggesting that the control system reliably detects incomplete or inadequate bisulfite conversion.

vi. Guard Banding

Several guard banding studies were conducted to evaluate target values and stable ranges for each reagent and process step (Tables 10 and 11).

Table 10. Summary of Results from Guard Banding Studies

Process	Parameter	Accepted Range	Target
Extraction	Lysis/Binding buffer vol	3.3-3.7 mL *	3.5 mL
Extraction	Plasma vol	3.0-4.0 mL *	3.5 mL
Extraction	Magnetic beads vol	80-100 µL	90 µL
Purification	Magnetic beads vol	18-22 µL	20 µL
Extraction	EtOH vol	2.3-2.7 mL *	2.5 mL
Extraction and Purification	EtOH in Wash A	45-55%	50%
Purification	Wash A vol bisDNA binding	900-110 µL	1000 µL
Purification	EtOH in Wash B	81-89%	85%
Extraction and Purification	Elution Tris concentration	5-15 mM	10 mM
Extraction and Purification	Elution pH	7.4-8.4 *	8.0
Extraction	Elution temperature	70-90 °C	80 °C
Purification	Elution temperature	18-28 °C *	23 °C
Extraction	Elution time	5-20 min	10 min
Purification	Elution Time	2-15 min *	10 min
Extraction	Elution volume	80-120 µL	100 µL
Purification	Elution volume	55-65 µL	60 µL
Extraction	Lysis time	5-15 min	10 min
All	Lysis/binding temperature	15-30 °C *	22 °C
Extraction	Binding time	30-60 min	45 min
Purification	Binding temperature	18-25 °C	22 °C
Purification	Binding time	30-60 min	45 min
Purification	Drying time	5-15 min	10 min
Purification	Drying temperature	18-25 °C	23 °C
Bisulfite Conversion	ABS solution pH	5.1-5.5	5.3
Bisulfite Conversion	TROLOX concentration	120-130 mg/mL	125 mg/mL
Bisulfite Conversion	Bisulfite solution volume	120-180 µL	150 µL
Bisulfite Conversion	Protection buffer volume	20-30 µL	25 µL
Bisulfite Conversion	Reaction temperature	77-83 °C	80 °C
Bisulfite	Reaction time	35-55 min	45 min

Process	Parameter	Accepted Range	Target
Conversion			
Storage	Plasma Quick Kit temp	-25 to -18 °C for 2d*	

* Statistically significant differences (p-value < 0.05) were detected, but mean delta Ct <1.

Table 11. Guard Banding Studies for the Epi proColon Sensitive PCR Kit

Parameter	<<	<	Target	>	>>	Result
PCR buffer conc	0.75x	0.85x	1x	1.15x	1.25x	P
PCR buffer pH	8.0	8.2	8.3	8.6	8.8	P*
$MgCl_2$ conc, mM	7.6	8.6	9.6	10.6	11.6	P
dNTPs conc, mM	0.3	0.4	0.5	0.6	0.7	P
Taq conc, U/µl	--	0.10	0.12	0.13	0.18	P
Primer Sept9 F8, µM	--	0.3	0.4	0.5	--	P
Primer Sept9 R, µM	--	0.3	0.4	0.5	--	P
Primer Sept9 B2, µM	--	0.8	1.0	1.2	--	P
Sept9-Taq-P5-DAB, µM	--	0.12	0.15	0.18	--	P
ACTB R, µM	--	0.08	0.10	0.12	--	P
ACTB F, µM	--	0.08	0.10	0.12	--	P
ACTB Taq TAMRA, µM	--	0.06	0.075	0.09	--	P
Activation time, min	--	15	20	25	--	P*
Activation temp, °C	--	92.5	94	95.5	--	P*
Break time, sec	--	1	5	6	--	P*
Break temp, °C	--	60.5	62	63.5	--	P*
Anneal time, sec	--	30	35	40	--	P*
Anneal temp, °C	--	54	55.5	57	--	P*
Denaturation time	--	20	30	40	--	P*
Denaturation temp	--	91.5	93	94.5	--	P*

P = passed, acceptance criteria met

* = Statistically significant difference

vii. <u>Specimen Stability</u>

Stability of plasma samples was evaluated under specified transport conditions to ensure that the transport and storage conditions were sufficient to maintain sample integrity (based on evaluation of mSEPT9 and ACTB) over a 5-year period. The data are available over a 42-month period. Blood samples from 36 patients with mSEPT9 CRC were processed to plasma, stored in aliquots at -80°C, and tested at four specified time points (0, 6-7, 31-34 and 39-42 months). For each time point, 2 plasma aliquots per patient were processed. In total, there were 288 observations (36 patients x 2 measurements x 4 time points).

There were no observable trends in reduction of positive calls or increases in Ct values over time based on the assay version. For samples with low target

concentrations, the positivity rate was similar across all time points. Two observations were called negative. In both cases, the results occurred for the first replicate for T1 and T3 while the second replicate was positive. Regression analyses of the Ct value data indicated that the time parameter had little to no effect on the study results. Taken together, the study results indicate that plasma samples stored up to 42 months at -80°C showed no evidence of degradation of mSEPT9 and ACTB. The time points tested in this study cover the duration that plasma samples were archived for the primary clinical study, Study 1.

viii. Intermediate Product Stability

The storage conditions for extracted DNA and bisulfite-treated DNA (BisDNA) were evaluated. Seven batches (2 for mode A and 1 for other modes) each containing 6 technical sample replicates were processed under six storage conditions using two lots per kit and one PCR instrument (Table 12). Each batch also contained 1 positive control and 1 negative control.

Table 12. Technical Sample Results for Intermediate Product Stability

Mode	Storage Condition		Batch QC	PDF
	Extracted DNA	BisDNA		Technical
A*	Used immediately	Used immediately	Valid Valid	6/6 6/6
B	> 18 hr at 2-8°C	> 18 hr at 2-8°C	Valid	6/6
C	> 24 hr at 2-8°C	> 24 hr at 2-8°C	Valid	6/6
D	> 72 hr at -25 to -15°C	> 72 hr at -25 to -15°C	Invalid	1/6
E	> 72 hr at -25 to -15°C	Used immediately	Invalid	4/6
F	Used immediately	> 72 hr at -25 to -15°C	Valid	6/6

* Standard workflow without interruption

Technical samples tested under modes A, B, C, and F met the acceptance criterion of 100% mSEPT9 positivity in valid runs. Batches tested under modes D and E were invalid and yielded reduced positive detection fractions (PDFs). Analyte degradation was due to storing extracted DNA for longer than 72 hours at -25°C to -15°C, since mode F did not yield a decrease in the PDF.

To further assess the storage conditions, the study was conducted for modes A, C and F using four CRC diluted plasma aliquots per mode (Table 13). Each batch contained 1 positive control and 1 negative control. Testing was conducted with one lot per kit and one PCR instrument. Batches tested under each condition were valid and yielded 100% mSEPT9 positivity.

Table 13. Plasma Sample Results for Intermediate Product Stability

Mode	Storage Condition		Batch QC	PDF
	Extracted DNA	BisDNA		Plasma
A*	Used immediately	Used immediately	Valid	4/4
C	> 24 hr at 2-8°C	> 24 hr at 2-8°C	Valid	4/4
F	Used immediately	> 72 hr at -25 to -15°C	Valid	4/4

* Standard workflow without interruption

The results indicate that extracted DNA may be stored up to 24 hours at 2°C to 8°C, and the purified bisulfite-treated DNA may be stored up to 24 hours at 2°C to 8°C or up to 3 days at -25°C to -15°C.

ix. Reagent Stability

a) Real-Time Stability Study

Two studies yielded consistent results to assess the shelf-life of the Epi proColon kits. In the first study, one validation lot for the three kits was tested at the following times: 0, 6, 12, 18, and 24 months. In the second study, two validation lots per kit were tested using one PCR instrument at the following time points: 0, 8, 9, 10, 11, 12, 13, 18, 19, 24, and 25 months. The two kit lots were subjected to additional shipping simulation conditions (Table 14). Each batch includes 8 samples (3 analyte positive, 3 analyte negative, 1 positive control, and 1 negative control).

Table 14. Transport Conditions Tested

	Kit	Condition	Kits per lot
0	Plasma Quick	Unstressed	10
1	Plasma Quick	35-39°C for 3 days, placed upside-down	10
2	Plasma Quick	3 freeze/thaw cycles	10
3	Sensitive PCR	5 days on dry ice	15
4	Control Kit	5 days on dry ice	20

Up to the time point at 13 months, 36 batches were processed and valid. At 8 months, one analyte positive sample was invalid due to a pipetting error and one analyte negative sample yielded a positive result. At 18 months, the Epi ProColon Plasma Quick Kit failed due to ineffective bisulfite solution. The acceptance criteria of 0.99 for the 95% confidence intervals of the proportions of valid results were met up to the 13 month time point.

The stability of the Epi ProColon Sensitive PCR Kit was demonstrated to be 18 months.

b) Isochronous Stability Study

An isochronous stability study for the kit reagents was performed to supplement the real-time stability data. Non-CRC and diluted CRC plasma samples were tested twice (8 week time lag between time points) using four kit combinations (Table 15). A total of seven replicates per sample were tested in eight batches. The acceptance criteria were as follows: 1) Results are in agreement with the real-time stability data (i.e., supports claims of 13 months for the Plasma Quick Kit and 18 months for the Epi ProColon Sensitive PCR Kit), and 2) the non-CRC samples show no statistically significant trend over time.

Table 15. Isochronous Stability Study Results

Batch	Lot Combination				Valid Results			
	Plasma Quick Kit		Sensitive PCR Kit		CRC plasma		Non-CRC plasma	
	Lot	Mo.	Lot	Mo.	Reps	PDF	Reps	PDF
1	1	6	1	5	7	7 (100%)	7	1 (14%)
2	2	11	2	10	7	7 (100%)	7	1 (14%)
3	1	6	3	20	7	7 (100%)	7	2 (29%)
4	2	11	4	28	7	7 (100%)	7	3 (43%)
5	1	8	1	7	7	7 (100%)	7	2 (29%)
6	2	13	2	12	7	7 (100%)	7	0 (0%)
7	1	8	3	22	7	7 (100%)	7	2 (29%)
8	2	13	4	30	6	6 (100%)	6	0 (0%)

All batches were valid, but two samples yielded invalid internal control results. A total of 55 (of 56) diluted CRC samples and 55 (of 56) non-CRC samples were analyzed. The observed positive detection fractions (PDFs) for the diluted CRC samples were 100% at every time point. For non-CRC samples, no statistically significant trend was observed over time using logistic regression models (kit age was independent factor). The results were consistent with the stability claims defined from the real-time stability studies.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Two clinical studies were initially conducted to support the clinical performance of the Epi proColon to screen adults of either sex, 50 years or older, defined as average risk for CRC, who are unable or unwilling to undergo routine CRC screening tests that are recommended by appropriate guidelines. Study 1 compared the performance of the Epi proColon test to colonoscopy. Study 2 compared the performance of the Epi proColon test and a fecal immunochemical test (FIT) to colonoscopy results. The clinical trials were performed in the US and Germany. Summaries of the clinical studies are presented below.

A. Study Design

i. Study Design for Study 1

Study 1 was a retrospective, multi-center clinical study to compare the performance of Epi proColon to that of colonoscopy. The primary objective was

detection of CRC by colonoscopy compared with the Epi proColon test result. Specifically, the objective was Epi proColon shall demonstrate sensitivity for CRC of 65% and specificity of 85% with statistical significance. Epi proColon shall also demonstrate valid results for at least 95% of clinical samples and valid test runs in at least 90% of standard runs. Secondary objectives were to evaluate test positivity in advanced adenoma (AA), small polyps (SP), and specimens from subjects with no evidence of disease (NED).

Subjects for the Study 1 were selected retrospectively from another trial titled, Prospective Evaluation of Septin 9 Performance in CRC Screening (PRESEPT, SPR0006) [3]. The PRESEPT study enrolled patients who were scheduled for screening colonoscopy from 22 practices in the US and 10 practices in Germany between June 2008 and January 2010. A total of 7941 subjects were enrolled in the PRESEPT study and a subset of 6857 subjects (all 53 colorectal cancers, 650 of 653 with advanced adenomas, 454 of 2369 with small polyps (< 10 mm without a villous component or high grade dysplasia and 469 of 3785 with no evidence of disease) were selected for evaluation in Study 1. The subjects were tested in a prospective evaluation prior to Study 1 using the first generation Epi proColon assay, which was based on 2 PCR replicates. In the prospective evaluation, results from 53 CRC cases and 1457 non-CRC cases yielded sensitivity and specificity of 50.9% and 91.4%, respectively [3].

In Study 1, samples were tested with the Epi proColon test at three laboratories, and sensitivity and specificity were defined using colonoscopy as the reference method, followed by histological confirmation when applicable. Based on colonoscopy results, enrolled participants were classified into the following four clinical groups:

i. CRC - Clinical/surgical diagnosis of invasive colorectal adenocarcinoma detected by optical colonoscopy and confirmed by histology for CRC cases (stages I-IV).

ii. AA including adenomatous polyp(s) equal to or greater than 10 mm, adenomas with a villous component or high grade dysplasia (HGD) as detected by colonoscopy and confirmed by histology.

iii. SP – polyps < 10mm and without a villous component or HGD.

iv. NED – no evidence of any of the above.

a) Clinical Inclusion and Exclusion Criteria

Inclusion and Exclusion Criteria for Enrollment in PRESEPT

Enrollment in Study 1 was limited to patients who met the inclusion criteria for a previous study (PRESEPT, SPR0006) as follows:

- Provide Informed Consent;

- Capable of providing adequate health history;
- Age 50 or older at time of colonoscopy (colorectal screening guideline-eligible);
- Accessible for blood draw prior to start of bowel preparation for colonoscopy;
- First colonoscopy in lifetime (or had flexible sigmoidoscopy more than 5 years prior to the scheduled colonoscopy).

Patients were not permitted to enroll in PRESEPT if they met any of the following exclusion criteria:

- Anorectal bleeding or hematochezia within last 6 months for which patient sought medical attention;
- Known iron deficiency anemia in the last 6 months for which patient sought or received medical attention;
- Previous history of colorectal polyps or CRC;
- High risk for CRC (2 or more primary relatives with CRC; 1 or more primary relative(s) < 50 years with CRC; known HNPCC or FAP).

Eligibility Criteria for Specimen Testing in Study 1

In Study 1, samples were excluded from testing if they met the following criteria:

- Exhibited gross hemolysis;
- Were non-compliant with the protocols for specimen collection, processing, storage, or shipping;
- Had inadequate volume for mSEPT9 analysis.

b) Follow-up Schedule

In the PRESEPT study, enrolled patients were scheduled for screening colonoscopy according to CRC screening guidelines at the time of the study. The results of the colonoscopy were used as the reference method to define diagnostic truth. The study sites were monitored, such that there were at least two visits during the specimen and data collection and one after data and specimen collection was completed.

Monitoring of the laboratories performing Epi proColon testing in Study 1 was conducted to determine if procedures for validation of the Epi proColon test were followed correctly and to inspect documentation and or processes at the testing site to ensure data accuracy. Monitoring visits were scheduled with the Investigator or designee for the site such that there was at least one visit during the conduct of the testing.

The test results from neither PRESEPT nor Study 1 were not used for patient management.

c) Clinical Endpoints

The criteria for valid test results and test runs were met, since 95.1% of the test results and 91.3% of the test runs were valid. With respect to the clinical performance, sensitivity for CRC of Epi proColon is 68.2% (95% CI: 53.4% – 80.0%). Removal of one 49 year old subject led to a slight decrease in sensitivity to 67.4% (95% CI: 51.3% – 80.4%). In the non-CRC subgroups – AA, SP, and NED – the specificity is 78.8% (95% CI: 76.7% – 80.8%).

Secondary objectives were to evaluate test positivity in specimens with AA, SP, and NED. The false positive fraction (or 1 – specificity) in these non-CRC groups was similar: 22% (95% CI: 19% – 25%) for AA; 20% (95% CI: 17% – 24%); and 22% (95% CI: 18% – 26%) for NED. Variation of the false positive fraction by non-CRC group was not significant (χ^2 test, 2 degrees of freedom, p-value = 0.76, significance level = 5%). The overall false positive fraction for all non-CRC groups was 21% (95% CI: 19% – 23%).

Post hoc subgroup analyses were also performed. Though interpretation should be taken with caution, the results indicate that age and ethnicity significantly affect the false positive fraction in non-CRC subjects, though not the true positive fraction (or sensitivity) in CRC subjects.

ii. <u>Study Design for Study 2</u>

Study 2 enrolled 336 patients between March 15, 2012 and November 19, 2012. There were 61 US enrollment sites and all testing was done at a single US testing site. Study 2 was a prospective, multi-center clinical study designed to compare the performance of Epi proColon and a fecal immunochemical test (FIT), OC FIT-CHEK (Polymedco, Inc.), to that of colonoscopy. The primary objective was to demonstrate non-inferiority in the clinical performance of Epi proColon to a commercially available FIT assay.

The performances of Epi proColon and a commercially available FIT were compared to colonoscopy results. Colonoscopy was used as the reference method. Clinical data, along with matched blood and stool specimens, were collected from each eligible subject. As in Study 1, subjects were classified into four clinical groups based on colonoscopy results: CRC, AA, SP and NED subjects. Subjects were then enrolled into one of the following two study arms:

- Group A - Patients have invasive CRC at screening colonoscopy (i.e., AJCC/UICC stages I, II, III, and IV). Collection of blood and stool occurred after colonoscopy, but prior to surgery or intervention.

- Group B - Subjects were prospectively enrolled and provided blood and stool samples prior to screening colonoscopy.

Groups A and B were set to have targeted quotas of 100 and 200 subjects, respectively, in order to estimate sensitivity and specificity. Blood samples for evaluation with Epi proColon were collected from each subject, processed to plasma, aliquoted, and shipped frozen to a central repository, where the samples were archived at -70°C. Stool samples for FIT testing were collected by the subjects using supplied kits and then shipped directly to one US testing laboratory, which conducted both Epi proColon and FIT tests.

a) Clinical Inclusion and Exclusion Criteria

Inclusion and Exclusion Criteria for Group A

Enrollment in Group A was limited to patients who met the following inclusion criteria:

- Willing and able to sign informed consent and to adhere to study requirements;
- 50-84 years of age at blood and stool sampling;
- Colonoscopic diagnosis or strong clinical suspicion of CRC. The suspected cases must have a confirmed diagnosis of CRC after surgery and be accompanied by a complete pathology report.
- Colonoscopy within 6 months before inclusion into the study;
- Blood and stool sampling a minimum of 10 days after colonoscopy and before resection surgery.

Patients were not permitted to enroll in Group A if they met any of the following exclusion criteria:

- Subjects with curative biopsy during colonoscopy;
- Previous personal history of CRC or previous colonoscopy resulting in a recommendation to repeat colonoscopy at an interval less than 10 years (i.e., high risk population);
- Neoadjuvant treatment;
- Familial risk for CRC (2 or more 1^{st} degree relatives with CRC; 1 or more 1^{st} degree relative(s) < 50 years with CRC; known HNPCC or FAP);
- History of inflammatory bowel disease;
- Acute or chronic gastritis;
- Current diagnosis of any other cancer than CRC;
- Overt rectal bleeding or bleeding hemorrhoids;
- Known infection with HIV, HBV or HCV;
- Concurrently receiving intravenous fluid at the time of sample collection.

Inclusion and Exclusion Criteria for Group B

Enrollment in Group B was limited to patients who met the following inclusion criteria:

- Willing and able to sign informed consent and to adhere to study requirements;
- 50-84 years of age at blood and stool sampling;

- Able to provide blood and stool sample prior to bowel preparation and colonoscopy.

Patients were not permitted to enroll in Group B if they met any of the following exclusion criteria:

- Previous personal history of CRC or previous colonoscopy resulting in a recommendation to repeat colonoscopy at an interval less than 10 years (i.e., high risk population);
- Neoadjuvant treatment;
- Familial risk for CRC (2 or more 1st degree relatives with CRC; 1 or more 1st degree relative(s) < 50 years with CRC; known HNPCC or FAP);
- History of inflammatory bowel disease;
- Acute or chronic gastritis;
- Current diagnosis of any other cancer than CRC;
- Overt rectal bleeding or bleeding hemorrhoids;
- Known infection with HIV, HBV or HCV;
- Concurrently receiving intravenous fluid at the time of sample collection.

b) Follow-up Schedule

Patients underwent screening colonoscopy. The results of the colonoscopy were used as the reference method to define diagnostic truth. Both Epi proColon and FIT were compared to colonoscopy results to establish clinical sensitivity and specificity. The results from the study were not used for patient management.

Data monitoring at the central testing lab was performed by Epigenomics and monitoring at the clinical sites was performed by a contract research organization. At least one monitoring visit occurred during the specimen and data collection phase.

c) Clinical Endpoints

The primary objective was to demonstrate non-inferiority in the clinical performance of Epi proColon to a commercially available FIT assay with non-inferiority margins for the differences in sensitivities and specificities of 0.1 and 0.2, respectively.

B. Accountability of PMA Cohort

i. Accountability of PMA Cohort for Study 1

In Study 1, a subset of 1623 archived specimens from the PRESEPT study (SPR0006) was evaluated (Table 16). In the PRESEPT study (SPR0006), a total of 7941 subjects were recruited from 32 sites, 22 in the US and 10 in Germany. Approximately 75% of the subjects were enrolled at US sites, and about 25% were enrolled at German sites. Among the enrolled subjects, 6857 met the eligibility criteria and had available specimens (Figure 1). All available CRC and AA subjects were selected for Study 1. Subsets of SP and NED subjects were selected using a stratified random sampling approach, such that (1) the 2010 US census demographic profile for age is represented in both subsets, (2) gender is represented equally, and (3) there is increased representation of ethnic minorities compared to the PRESEPT study (see section titled “Study Population Demographics for Study 1”).

Figure 1: Subject Accountability in the PRESEPT Study

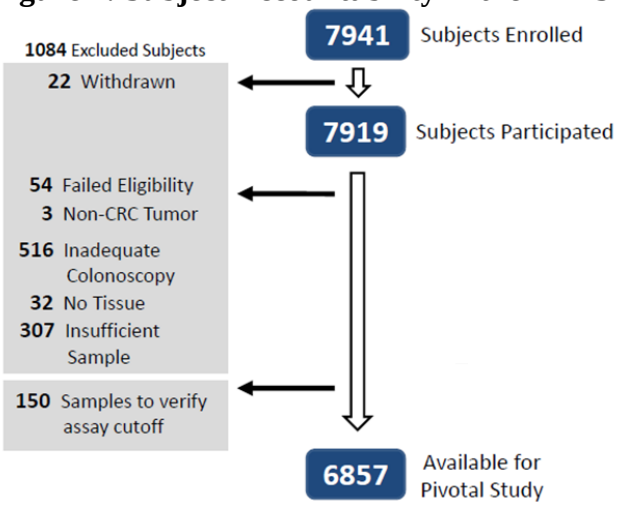


Figure 2: Subject Selection for Study 1

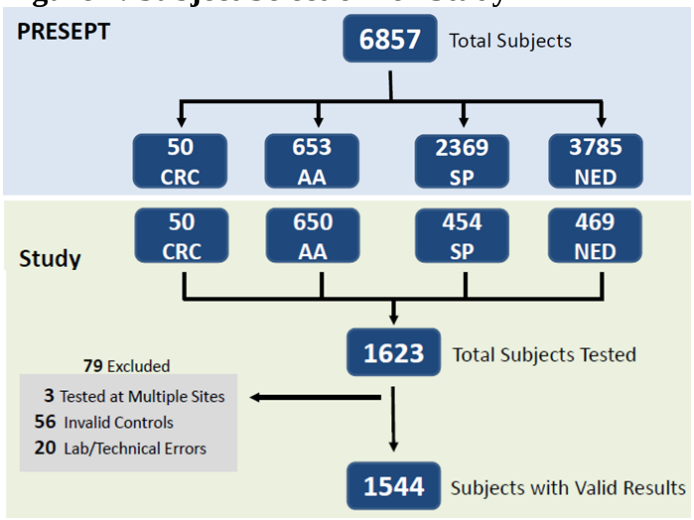


Table 16. Subjects Selected for Study 1

Clinical Group	PRESEPT	Study 1	
	Available Subjects	Tested Subjects	Valid Test Results
CRC	50	50	44
AA*	653	650	621
SP	2369	454	435
NED	3785	469	444
Total	**6857**	**1623**	**1544**

* Plasma specimens were not located for three subjects from the PRESEPT study.

In total, valid Epi proColon results were obtained for 1544 of the 1623 samples evaluated (Table 16 and Figure 2); 79 samples were excluded due to invalid external process controls (n=43), invalid internal control (n=13), distribution of sample to more than one testing site (n=3), and technical errors (n=20). Among

the 134 batches processed, eight were invalid due to improper functioning of the PCR instrument, invalid controls, and handling errors. Valid results were reported for 115 batches (91.3%). The number of subjects that were excluded from Study 1 and the reason for exclusion can be found in Table 17.

Table 17. Subjects Excluded from Study 1

Subjects	CRC	AA	SP	NED	Total
Total number of subjects tested	50	650	454	469	1623
Excluded due to invalid process controls (no result reported and repetition not possible)	5	19	6	13	43
Excluded due to invalid internal control (ACTB failure)	0	4	4	5	13
Excluded due to errors in batch assembly (samples sent to multiple sites)	0	0	2	1	3
Excluded due to documented laboratory or technical errors	1	6	7	6	20
Total number of subjects excluded	6	29	19	25	79
Total number of subjects with valid result	44	621	435	444	1544

ii. Accountability of PMA Cohort for Study 2

A total of 337 subjects were enrolled prospectively from 61 US sites for Study 2. Thirty-six subjects did not meet the inclusion/exclusion criteria (Table 18), resulting in 301 remaining subjects.

Table 18. Subjects Excluded from Study 2

Reason	Group A	Group B	Total
Incomplete data	0	1	1
Curative biopsy	3	0	3
Did not meet age requirement	2	0	2
Invalid Epi proColon and missing FIT result	1	0	1
Neoadjuvant therapy	3	1	4
No colonoscopy	0	17	17
No samples available	2	1	3
Not average risk	2	3	5
Total	13	23	36

All remaining 301 subjects provided plasma samples and 290 provided stool samples that were evaluable (Table 19). Stool samples from 11 patients were not available for the following reasons: eight patients did not provide stool samples prior to scheduled colonoscopy, and three additional subjects did not have their samples tested within the pre-specified timeframe.

Table 19. Study Samples for Study 2

Clinical Group	Group A		Group B		Total		
	Blood	Stool	Blood	Stool	Blood	Stool	
					Included	Included	Excluded
CRC	99	95	2	2	101	97	4
AA	3	2	26	25	29	27	2
SP	--	--	77	75	77	75	2
NED	--	--	94	91	94	91	3
Total	102	97	199	193	301	290	11

Group A CRC subjects (n=102) were consecutively enrolled from an average-risk, screening guideline-eligible population who underwent colonoscopy and who have been diagnosed with, or where there was strong clinical suspicion of, CRC. Upon pathological review, three subjects were diagnosed with AA while all others had CRC. Group B subjects (n=199) were consecutively enrolled from an average-risk, symptom-free population who were able to provide blood and stool samples prior to colonoscopy.

C. Study Population Demographics and Baseline Parameters

i. Study Population Demographics for Study 1

In Study 1, a subset of specimens from the PRESEPT study (SPR0006) was evaluated. All available CRC and AA subjects were selected. Subsets of SP and NED subjects were selected using a stratified random sampling approach, such that (1) the 2010 US census demographic profile for age is represented in both subsets, (2) gender is represented equally, and (3) there is increased representation of ethnic minorities compared to the PRESEPT study (Table 20).

Table 20. Demographic Distribution for Study 1 Subjects

Factor	Stratum	CRC % (n)	AA % (n)	SP % (n)	NED % (n)	Total %
Gender	Female	32 (14)	43 (267)	51 (221)	50 (223)	47
	Male	68 (30)	57 (354)	49 (214)	50 (221)	53
Age	50-59	9 (4)	35 (218)	45 (195)	45 (198)	40
	60-69	55 (24)	47 (294)	30 (131)	29 (127)	37
	> 69	36 (16)	18 (109)	25 (109)	27 (119)	23
Ethnicity	Caucasian	89 (39)	85 (527)	66 (288)	63 (278)	73
	African-American	7 (3)	9 (56)	21 (92)	25 (110)	17
	Others*	5 (2)	6 (38)	13 (55)	13 (56)	10
Country	USA	59 (26)	77 (480)	84 (365)	84 (373)	81
	Germany	41 (18)	23 (141)	16 (70)	16 (71)	19

* Includes Hispanic, American Indians, Alaska Natives, Asians, Native Hawaiians, Other Pacific Islanders, and unclassified others.

ii. Study Population Demographics for Study 2

The demographics of the study population are summarized in Table 21.

Table 21. Demographic Distribution for Study 2 Subjects

Factor	Stratum	Group A % (n)	Group B % (n)
Gender	Female	32% (33)	61% (122)
	Male	68% (69)	39% (77)
Age	50-59	25% (25)	64% (127)
	60-69	37% (38)	25% (49)
	> 69	39% (40)	12% (23)
Ethnicity	Caucasian	69% (70)	70% (140)
	African-American	11% (11)	14% (27)
	Hispanic	17% (17)	12% (24)
	Other	4% (4)	4% (8)

D. Safety and Effectiveness Results

i. Safety Results

The analysis of safety was based on both studies. No adverse effects were reported.

ii. Effectiveness Results

The analyses of effectiveness based on sensitivity and specificity were from clinical studies Study 1 and Study 2. Key outcomes are presented in Tables 22 to 24 for VAL0018 and in Tables 25 to 27 and Figure 3 for Study 2.

a) Effectiveness Results for Study 1

Valid test results were assessed to determine the clinical performance of Epi proColon in terms of sensitivity for CRC and specificity in subjects without CRC (Table 22). The sensitivity of Epi proColon is 68.2% (95% CI: 53.4% – 80.0%). The sensitivity decreased to 67.4% (95% CI: 51.3% – 80.4%) upon removal of one 49-year old subject. In the non-CRC subgroups – AA, SP, and NED – the specificity is 78.8% (95% CI: 76.7% – 80.8%).

Table 22. Study 1 Results

		Epi proColon		
		Negative	**Positive**	**Total**
Colonoscopy	**CRC**	14	30	44
	Non-CRC	1182	318	1500
	Total	1196	348	1544
*Sensitivity		68.2% (95% CI: 53.4% – 80.0%)		
Specificity		78.8% (95% CI: 76.7% – 80.8%)		

* This includes one 49 year old subject, who had CRC by colonoscopy and a positive Epi proColon result. The estimated sensitivity without this subject is 67.4% (95% CI: 51.3% – 80.4%).

Since the study population was enriched with CRC and AA cases, compared to SP and NED cases, adjusted predictive values were weighted according to the prevalence of each disease category that was observed in the PRESEPT study (Table 23). As listed in Table 23, the prevalence for CRC was 0.7%, 9.5% for AA, 34.6% for SP, and 55.2% for NED in the PRESEPT study. The positive predictive value indicates that the probability of CRC among those patients that test positive was 2.3%. On the other hand, for those who test negative, the probability of CRC was 0.3% (i.e., probability of not having CRC was 99.7%). The positive predictive value indicates that among patients who test negative, the probability of having AA, SP or NED is, respectively, 9.5, 35.2 and 55%. Each of these is similar to the prevalence of AA, SP and NED, respectively. Point estimates and 95% CIs were computed by bootstrap methodology and in a Bayes formula for predictive values.

Table 23. Adjusted Predictive Values from Study 1

Prevalence in PRESEPT (n=6857)	**Point Estimate**	**Parameter**	**Point Estimate**	**95% CI**
CRC	0.7%	Positive Predictive Value	2.3%	1.8% – 2.9%
Non-CRC	99.3%	Negative Predictive Value	99.7%	99.6% – 99.8%
AA	9.5%	P(AA|negative)*	9.5%	9.1% – 9.9%
SP	34.6%	P(SP|negative)†	35.2%	33.8% – 36.7%
NED	55.2%	P(NED|negative) ‡	55.0%	53.4% – 56.5%

* Probability of AA given a negative test result
† Probability of SP given a negative test result
‡ Probability of NED given a negative test result

The False Positive Fraction for each non-CRC subgroup range from 20% to 22% (Table 24). Variation of the false positive fraction by non-CRC group was not statistically significant (χ^2 test, 2 degrees of freedom, p-value = 0.76,

significance level = 5%) indicating that positive results do not identify people with significantly higher risk for AA or SP.

Table 24. False Positive Fraction (FPF) in Non-CRC Groups in Study 1

Non-CRC Group	Epi proColon			
	Positive	Total	FPF	95% CI
AA	134	621	22%	19% – 25%
SP	87	435	20%	17% – 24%
NED	97	444	22%	18% – 26%
Total	318	1500	21%	19% – 23%

b) Effectiveness Results for Study 2

The sensitivity and specificity for CRC with Epi proColon and FIT, as well as non-inferiority of Epi proColon to FIT, were evaluated in Study 2. The results from the matched blood and stool samples are listed in Tables 25 and 26.

Table 25. Results for Epi proColon and Colonoscopy in Study 2

		Epi proColon		
		Positive	Negative	Total
Colonoscopy	CRC	70	27	97
	Non-CRC	37	156	193
	Total	107	183	290
Sensitivity		72.2% (95% CI: 62.5% – 80.1%)		
Specificity		80.8% (95% CI: 74.7% – 85.8%)		

Table 26. Results for FIT and Colonoscopy in Study 2

		FIT		
		Positive	Negative	Total
Colonoscopy	CRC	66	31	97
	Non-CRC	5	188	193
	Total	71	219	290
Sensitivity		68.0% (95% CI: 58.2% – 76.5%)		
Specificity		97.4% (95% CI: 94.1% – 98.9%)		

The difference in sensitivity between FIT and Epi proColon is -4.2% (95% CI: -16.2%, 8.1%), in favor of Epi proColon. The difference in specificity is 16.6% (95% CI: 10.6%, 22.9%), in favor of the FIT test.

Positive and negative diagnostic likelihood ratios (DLRs) for Epi proColon and FIT were determined and compared (Table 27). The positive DLR was

3.76 for Epi proColon and 26.26 for FIT, indicating that a Epi proColon positive test result was 3.76 times more likely for a subject with CRC than a subject without CRC, while a FIT positive test result was 26.26 times more likely. This increase of 22.5 was statistically significant (95%CI: 9.45 – 127.40) and implies that the positive predictive value (PPV) is greater with FIT than with Epi proColon, for the same prevalence of CRC (because PPV is a monotone increasing function of positive DLR).

The negative DLR is 0.34 for Epi proColon and 0.33 for FIT, indicating that a Epi proColon negative test result was 0.34 times as likely for a subject with CRC than a subject without CRC, while a FIT negative test result was 0.33 times as likely. The difference of 0.01 (95%CI: -0.16 – 0.12) was not significant. Essentially, negative DLR results are comparable for FIT and Epi proColon, implying that the two tests have comparable negative predictive values, for the same prevalence of CRC (because NPV is a monotone decreasing function of negative DLR).

Table 27. Comparison of Diagnostic Likelihood Ratios from Study 2

Metric	Epi proColon	FIT	Difference (95% CI)
Positive DLR*	3.76	26.26	22.5 (9.45 – 127.40)
Negative DLR†	0.34	0.33	-0.01 (-0.16 – 0.12)

* Positive DLR = sensitivity / (1-specificity)
† Negative DLR = (1-sensitivity) / specificity

Non-parametric Receiver Operating Characteristic (ROC) curves for the pairs of sensitivity and false positive fraction (1-specificity) using all possible values of the positivity threshold for FIT and Epi proColon were generated (Figure 3). The FIT score was considered for the FIT ROC curve, and the curve for Epi proColon uses the number of positive test results (among the triplicate wells). In Figure 3, the black dot and the blue dot depict the true positive fraction and false positive fraction pair based on the pre-specified positivity threshold for FIT and Epi proColon, respectively. The positivity threshold for Epi proColon is such that the test is considered positive if at least 1 of the 3 PCR replicate wells is positive. The ROC plot for Epi proColon was generated by varying the minimum number of positive wells to yield a positive test result. Whereas the ROC plot for FIT was obtained by varying the FIT score. The area under the ROC curve (AUC) for FIT is greater than the one for Epi proColon (0.86 vs. 0.82), although the difference in AUC of 0.04 is not significant (95%CI: -0.02 – 0.11).

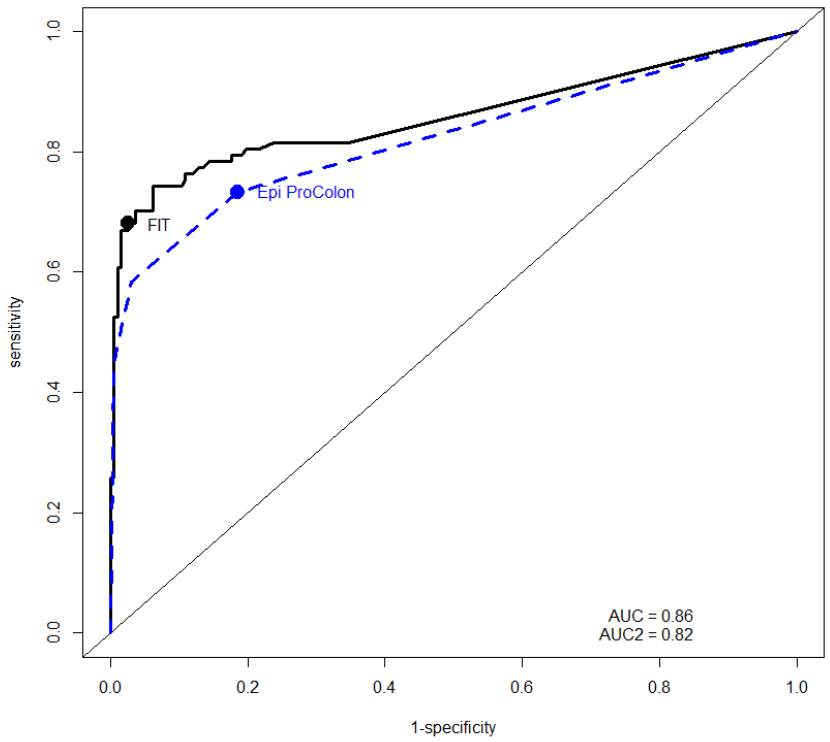


Figure 3. ROC plot for sensitivity (true positive fraction) vs. 1-specificity (false positive fraction) for FIT (black solid line) and for Epi proColon (blue dashed line) over all positivity thresholds. Superimposed in the ROC plot are the coordinates (sensitivity, 1-specificity) based on the pre-specified positivity threshold for FIT and Epi proColon, respectively.

c) Subgroup Analyses

Post hoc subgroup analyses were performed for Study 1. These analyses were interpreted with caution since the study was not designed to evaluate the performance of the test in subgroups. In addition, no attempt was made to adjust for multiplicity.

The false positive fraction (FPF) was evaluated by age group in non-CRC subjects. An increase was observed with increasing age from 16% to 26%, which suggests that specificity of Epi proColon decreases with increasing age (Fisher-Freeman-Halton test, 2-sided exact p-value < 0.001) (Table 28). Similar analysis shows no evidence of significant differences in true positive fraction (TPF) for CRC subjects across the same age categories (p-value = 1.0).

Table 28. Results by Diagnosis and Age in Study 1

Age	CRC subjects				Non-CRC Subjects			
	Neg	Pos	TPF	95% CI	Neg	Pos	FPF	95% CI
50-59	1	3	75.0%	30.1% – 98.7%	511	100	16.4%	13.6% – 19.5%
60-69	8	16	66.7%	46.7% – 82.0%	422	130	23.6%	20.2% – 27.3%
> 69	5	11	68.8%	44.4% – 85.8%	249	88	26.1%	21.7% – 31.1%

Since subjects were enrolled in the US and Germany, an additional analysis by site was conducted (Table 29). In CRC subjects, sensitivity estimates for subjects from the US and Germany were 57.7% and 83.3% respectively. Although the difference in sensitivity between sites appears large, it was not statistically significant (p=0.10). In non-CRC subjects, specificity estimates for subjects from the US and Germany were 78.6% and 79.8% respectively. The difference in specificity between sites was not statistically significant (p=0.69).

Table 29. Study 1 Results by Site

		Epi proColon					
		US			Germany		
		Neg	Pos	Total	Neg	Pos	Total
Colonoscopy	CRC	11	15	26	3	15	18
	Non-CRC	957	261	1218	225	57	282
	Total	968	276	1244	228	72	300
Sensitivity (95% CI)		57.7% (38.9% – 74.5%)			83.3% (60.8% – 94.2%)		
Specificity (95% CI)		78.6% (76.2% – 80.8%)			79.8% (74.7% – 84.1%)		

d) Diagnostic-Yield in a Screening Population (Projected)

In a hypothetical screening population of 100,000 subjects, Epi proColon was evaluated relative to FIT on diagnostic yield for CRC based on the Study 2 results and assuming CRC prevalence is 0.7% (Table 30). Among 100,000 screened subjects, numbers of subjects with true positive (TP), false negative (FN), false positive (FP), and true negative (TN) results were projected for each test. Projections indicate the number of FP subjects observed before CRC was detected in one subject (i.e., a TP subject was observed) would be on average 37.7 for Epi proColon and 5.4 for FIT. Projections also indicate that Epi proColon would on average detect one more TP subject than FIT at the expense of 571 more FP subjects. The results were not evaluated for statistical significance.

Table 30: Comparison of Epi proColon with FIT on Diagnostic Yield for CRC in a Hypothetical Screening Population of 100,000 Subjects (CRC prevalence 0.7%)

	700 CRC cases		99,300 non-CRC cases		False Positive per True Positive
	True Positive	False Negative	False Positive	True Negative	
Epi proColon	505	195	19,037	80,263	37.7
FIT	476	224	2,573	96,727	5.4
Difference	29	-29	16,464	-16,464	
Difference / 29	1	-1	571	-571	

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

A. Study Design for Study 3

Study 3 was conducted based, in part, on recommendations from the advisory committee with the aim to evaluate adherence to Epi proColon in a study population that have a history of non-compliance with an established CRC screening program. The goal of the study was to determine if Epi proColon would favorably affect patients' participation and compliance with an established CRC screening program. The study results were used to inform the intended use for Epi proColon, with the device "indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC, who have been offered and have a history of not completing CRC screening. Tests that are available and recommended in the USPSTF 2008 CRC screening guidelines should be offered and declined prior to offering the Epi proColon test."

Study 3 was a prospective, multi-center, two-arm clinical study performed under IDE G140155. This study enrolled 490 subjects (413 eligible) from 2 US sites between December 5, 2014 and March 24, 2015. Eligible men and women, who had at least two CRC screening recommendations in the past and were not up-to-date, were randomized to the Epi proColon blood test or a FIT test (OC FIT-CHEK, Polymedco, Inc.). The study compared adherence to CRC screening for Epi proColon and the FIT test to determine if Epi proColon could be declared not unacceptable relative to FIT

in terms of negative likelihood ratios adjusted for adherence, if the adherence-adjusted negative likelihood ratio for EPC is better than that of FIT, which would occur if the difference in adherence is greater by a certain margin. The adherence margin that was required for the adherence-adjusted negative likelihood ratio to be superior for Epi proColon compared to FIT was based on the assumption that adherence led to a valid test result. The study also evaluated the compliance to colonoscopy for subjects with positive test results from either Epi proColon or FIT, and the diagnostic yield (findings by colonoscopy) for the two tests.

i. Inclusion and Exclusion Criteria

Enrollment in Study 3 was limited to patients who met the following inclusion criteria:

- 50 years of age or greater, but less than 76 years old;
- Has not completed recommended screening for colonoscopy or FIT;
  - No colonoscopy in previous 10 years;
  - No Fecal occult blood test or FIT in previous year and/or 13 months late for FIT;
- No flexible sigmoidoscopy in previous 5 years;
- Verifiable offer of screening recommendation according to health system standard in at least two independent interaction and verifiable lack of adherence for two most recent;
- Verifiable lack of adherence for > 3 months following last screening recommendation;
- Primary care physician has agreed to refer patients for consideration of enrollment in the study;
- Subject able to understand and sign written informed consent.

Patients were not permitted to enroll in Study 3 if any of the following exclusion criteria was met:

- Family history of CRC in a first-degree relative;
- Personal history of colonic adenomatous polyps, CRC or inflammatory bowel disease;
- Symptoms for which colonoscopy or sigmoidoscopy would otherwise be performed (hematochezia, new onset diarrhea or constipation, abdominal pain);
- Chronic gastritis, pregnancy, cancer(s) other than CRC.

ii. Follow-up Schedule

Screening test results were made available to the patients within 14 days of test completion. Subjects with a negative FIT or Epi proColon test received a letter providing the result, recommendations for continued screening and contact information for further counseling. Subjects with a positive FIT or Epi proColon result were contacted by phone by the health care provider or designee to inform them of results and provide them with counseling and provide a recommendation

to schedule a colonoscopy. Additionally, subjects could be contacted by mail, to inform them that their screening test was positive and counseling them of the follow-on steps for colonoscopy. Research staff recorded the scheduling and completion of colonoscopies.

iii. Clinical Endpoints

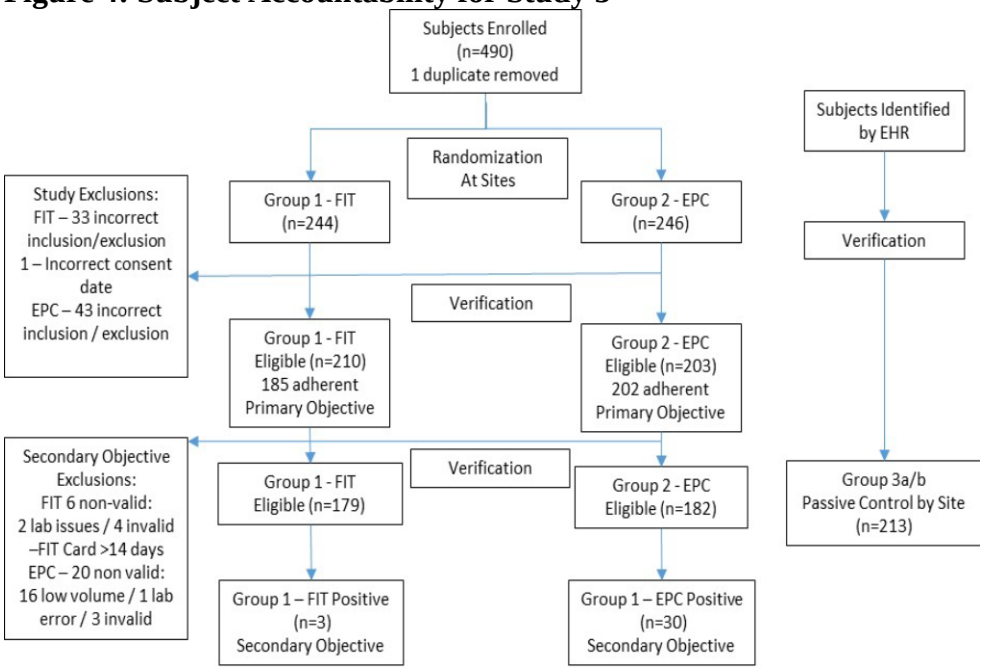
Primary objective:
The primary endpoint of Study 3 was to demonstrate that Epi proColon (EPC) could be declared not unacceptable relative to FIT in terms of negative likelihood ratios adjusted for adherence, if the adherence-adjusted negative likelihood ratio (ANLR) for EPC is better than that of FIT, which would occur if the difference in adherence is greater by a certain margin (pre-specified at 8.18775%).

Secondary objectives:
Descriptive goals were to determine the rate of compliance to follow-up colonoscopy for the two tests, given that a subject's test result was positive, and to determine the diagnostic yield (number of abnormal findings as determined by colonoscopy) for the two tests.

B. Accountability of PMA Cohort for Study 3

A total of 490 subjects were enrolled prospectively at two sites in the US. Seventy-six (76) subjects were excluded because they did not meet the inclusion/exclusion criteria, and 1 subject was excluded due to incorrect consent data. Of the remaining 413 subjects, 203 were assigned the Epi proColon arm and 210 were assigned to the FIT arm. For the 203 subjects who were assigned to the Epi proColon arm, 20 had invalid results. The reported causes for the invalid test results for Epi proColon were insufficient blood draw (n=16), laboratory error (n=1), and invalid result after completion of the assay (n=3). For the 210 subjects that were assigned to the FIT arm, 6 had invalid results. The reported causes for the invalid test results for FIT were laboratory issues (n=2) and delay in the receipt of a FIT card after sample collection (n=4) (Figure 4).

Figure 4: Subject Accountability for Study 3



C. <u>Study Population Demographics for Study 3</u>

The demographics of the study population are summarized in Table 31.

Table 31. Demographic Distribution of Study 3 Subjects

Factor	Stratum	FIT % (n)	Epi proColon % (n)
Gender	Female	60% (126)	61% (123)
	Male	40% (84)	39% (80)
Age	50-59	51% (107)	58% (117)
	60-69	42% (88)	35% (72)
	> 69	7 % (15)	7% (14)
Ethnicity	Native American	0.5% (1)	0% (0)
	Asian	0.5% (1)	1% (3)
	African-American	8% (17)	7% (15)
	Caucasian	86% (180)	83% (174)
	Hispanic	9% (4)	5% (10)
	Native Hawaiian	1% (2)	0% (0)
	Other	0% (0)	0.5% (1)

D. Safety and Effectiveness Results for Study 3

No adverse effects were reported.

Adherence to screening was calculated based on all samples obtained from patients for testing regardless of the validity of test results. Two hundred ten (210) subjects were randomized to the FIT arm, of which 185 completed testing for an adherence rate of 88.1% (95% CI: 83.0% – 91.8%) (Table 32). In the Epi proColon arm, 203 subjects were randomized, of which 202 completed blood testing for an adherence rate of 99.5% (95% CI: 97.3% to 100%). The difference in adherence was 11.4% with a 95% CI of 6.9% to 15.9% (one-sided p-value=0.059).

Table 32. Adherence to Screening with Epi proColon and FIT

Study Arm	Adherence	Site 1 (n)	Site 2 (n)	Total (n)	Adherence Rate (95% CI)
FIT	**Adherent**	72	113	185	88.10% (83.0% – 91.8 %)
	Non-Adherent	12	13	25	
	Total	84	126	210	
Epi proColon	**Adherent**	85	117	202	99.5% (97.3% – 100 %)
	Non-Adherent	1	0	1	
	Total	86	117	203	

The invalid assay rate was higher for Epi proColon compared to FIT. Invalid test results were obtained in 9.9% (20/203) of the patients assigned to Epi proColon as compared to 3.2% (6/185) of the patients assigned to FIT. When invalid results are incorporated into the primary analysis and are treated as non-adherent, the following results are obtained: 89.7% (182/203) EPC adherence vs. 85.2% (179/210) FIT adherence. The adherence difference was 4.5 (95% CI: -2.0% –10.8%).

A secondary endpoint was to determine the rate of compliance to follow-up colonoscopy for the two tests, given that a subject's test result was positive. For Epi proColon, 17 of 30 patients with a positive result went on to receive a colonoscopy. For FIT, 1 of 3 patients with a positive result went on to receive a colonoscopy.

Another secondary endpoint was to determine the diagnostic yield (colonoscopy output) for the two tests. None of the follow-up colonoscopy tests detected any cancers. For patients who received a colonoscopy after a positive Epi proColon test, 10/17 were found to have polyps or lesions. For the 1 patient who received a colonoscopy after a postive FIT test, 1/1 was found to have polyps or lesions.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

A. Panel Meeting Recommendation

At an advisory meeting held on March 26, 2014, the Molecular and Clinical Genetics Panel voted (9 yes, 0 no, and 1 abstain) there is reasonable assurance the device is safe, (5 yes and 6 no) there is not reasonable assurance that the device is effective, and (5 yes, 4 no and 1 abstain) that the benefits of the device do outweigh the risks in patients who meet the criteria specified in the proposed indication.

The panel recommended that the test be indicated in a second-line setting, and that a post-approval study be performed to evaluate the programmatic performance of Epi proColon.

The panel meeting materials can be found at the following website: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/MolecularandClinicalGeneticsPanel/ucm390219.htm.

B. FDA's Post-Panel Action

Based, in large part, on recommendations from the advisory committee, Epigenomics conducted Study 3 to assess adherence to screening with the Epi proColon test compared to screening with a FIT test. The goal of Study 3 was to determine if Epi proColon would favorably affect patients' participation and compliance with an established CRC screening program. In addition, per the panel's recommendations, the intended use, including contraindications and warnings, was modified to indicate that the Epi proColon test is not intended for patients who are willing and able to undergo routine CRC screening tests that are recommended by appropriate guidelines. Also, patient and physician brochures were revised to describe the performance of Epi proColon compared to FIT such that users will be appropriately informed and educated about the device. Panel recommendations were considered, and terms related to the performance of Epi proColon and recommended CRC screening methods (i.e., FIT) - such as sensitivity, specificity, positive predictive value, and negative predictive value - were defined to educate and allow the patient to

be properly informed when making a decision, in consultation with their healthcare provider, on whether or not to take the test.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

In Study 1, Epi proColon showed 68.2% (95% CI: 53.4% – 80.0%) sensitivity and 80.0% (95% CI: 77.9% –82.1%) specificity when using colonoscopy results as clinical truth. In Study 2, Epi proColon showed 73.3% (95% CI: 63.9% – 80.9%) sensitivity and 81.5% (95% CI: 75.5% – 86.3%) specificity when using colonoscopy results as clinical truth . The Epi proColon test demonstrated inferiority to a fecal test (OC FITCHEK, Polymedco, Inc.) for specificity, indicating that the Epi proColon test exhibited a higher rate of false positive results compared to the FIT test. The Epi proColon demonstrated non-inferiority to a fecal test for sensitivity indicating similar rate of positive results for people who have colorectal cancer.

Study 3 demonstrated 99.5% of the study population underwent screening with Epi proColon. It was expected that the population from Study 3 would have a relatively low adherence to FIT since the enrollment criteria specified that patients had to be non-compliant for CRC screening and declined at least 2 previous recommendations to be screened from their health care provider. Since the study achieved higher-than-expected adherence to FIT, with 88.1% of subjects completing the test, the estimates from this study may not accurately reflect the expected adherence rate to Epi proColon in the intended population not willing or able to undergo recommended screening tests. However, the results from Study 3 demonstrate that participants were willing to take Epi proColon at sufficiently high rates. Therefore, there is reasonable assurance that some patients who have been offered and have failed to undergo recommended CRC screening tests will be willing to take Epi proColon.

The performance of Epi proColon has been established in cross-sectional (i.e., single point in time) studies. Programmatic performance of Epi proColon (i.e., benefits and risks with repeated testing over an established period of time) was not established.

B. Safety Conclusions

The risks of the device are based on non-clinical laboratory data, as well as data collected in clinical studies conducted to support PMA approval, as described above. Erroneous device results could delay detection of CRC due to false negative results. False positive results could lead to an increased number of colonoscopies and associated adverse events. Patients who would otherwise select another CRC screening option with performance advantages may not be appropriate for this device. Patients who are tested by this device are subject to peripheral blood specimen collection, which is a standard procedure in clinical care and considered to be minimal risk.

C. Patient Perspective information

Patient perspective information was considered during the review. Specifically, a patient representative was included in the March 26, 2014 Advisory Panel meeting that convened to discuss Epi proColon. In addition, Epi proColon provides an option for adults of either sex, 50 years or older, defined as average risk for CRC, who are unable or unwilling to undergo routine CRC screening tests that are recommended by appropriate guidelines.

D. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in clinical studies conducted to support PMA approval, as described above. The benefits include that this is a non-invasive blood test that can be used to test adults of either sex, 50 years or older, defined as average risk for CRC, who are unable or unwilling to undergo routine CRC screening tests that are recommended by appropriate guidelines. This may lead to an increase in compliance rates to CRC screening programs in this intended population.

A risk of this test is that the convenience of it may reduce the willingness for people to undergo the recommended screening tests (i.e., colonoscopy or FIT) in favor of the Epi proColon test. This risk was mitigated in the labeling including a revised intended use statement and requiring patient brochures that describe the performance of the test in easy to understand manner. The risks of false positive results for patients to be referred for colonoscopy are mitigated since colonoscopy is a preferred CRC screening alternative. An additional risk is that the performance of Epi proColon has not been evaluated for repeat use. The post-approval study will be conducted to evaluate the repeat performance of Epi proColon (see Section E below), and additional warnings in the current labeling mitigate this risk.

In conclusion, given the available information, the data support that for testing adults of either sex, 50 years or older, defined as average risk for CRC, who are unable or unwilling to undergo routine CRC screening tests that are recommended by appropriate guidelines, the probable benefits outweigh the probable risks for this device.

E. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. CRC is the third most common cancer and the second leading cause of cancer-related deaths in the US [4]. CRC screening reduces deaths from CRC; however, about one-third of the average risk population remains unscreened for CRC [5]. Epi proColon provides an option for adults of either sex, 50 years or older, defined as average risk for CRC, who are unable or unwilling to undergo routine CRC screening tests that are recommended by appropriate guidelines, and therefore may help increase compliance for CRC screening.

Uncertainties remain regarding the programmatic performance of Epi proColon. For that reason, a post approval study will evaluate longitudinal performance of Epi proColon with respect to test positivity, longitudinal adherence to Epi proColon screening, adherence to follow-up colonoscopy and diagnostic yield, as well as assay failure rates. These results will be used to update the labeling as appropriate.

XIV. CDRH DECISION

CDRH issued an approval order on April 12, 2016. The final conditions of approval cited in the approval order include performing a post-approval study (PAS). The Epi proColon PAS will be a single arm, prospective, longitudinal, multi-center study to evaluate the performance of the device upon repeat use (i.e., second screening one year later, T1) in patients of average risk for CRC who, after appropriate counseling from their healthcare provider, have been offered and declined screening by USPSTF recommended methods. It is expected that 4,500 study participants will be enrolled. The primary endpoints are: (1) the proportion of participants at T1 with a positive test result, but without colorectal cancer, is significantly less than the proportion of subjects at the first screening (T0) with a positive test result, but without colorectal cancer; and (2) the test detects CRC at T1 in patients tested negative at T0. The secondary endpoints are: (1) the cumulative probability of cancer detection; (2) the cumulative probability of a false referral; (3) the probability of testing negative at both time points; (4) the adherence to Epi proColon at both screenings (i.e., T0, T1); (5) the diagnostic yield; (6) the adherence to follow-up diagnostic colonoscopy after a positive Epi proColon test; and (7) the assay failure rate.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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