FDA Executive Summary

Prepared for the
March 26, 2014 meeting of the
Molecular and Clinical Genetics Panel

P130001
Epi proColon®
Epigenomics AG

INTRODUCTION

This document is the **FDA Executive Summary** for the premarket approval (PMA) application P130001 from Epigenomics AG for Epi proColon. The device is a qualitative assay for the real-time PCR detection of methylated Septin9 DNA in human plasma, and is intended to screen patients for colorectal cancer, who are defined as average risk by current screening guidelines. The submission is under review by the Division of Immunology and Hematology Devices (DIHD), Office of *In vitro* Diagnostics and Radiological Health (OIR), within the Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA).

This document will summarize FDA's review of the PMA, highlighting the areas for which FDA seeks expertise and input from the Molecular and Clinical Genetics Panel. These topics will include the device performance and clinical experience to date. There are currently no devices approved in the United States for the proposed intended use. The Panel is being convened to discuss the potential benefit versus risk of using Epi proColon in the context of the proposed intended use and to offer advice on the degree to which the clinical validation data demonstrate safety and effectiveness in support of PMA approval for this "first of a kind" device.

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1 PROPOSED INTENDED USE

The Sponsor has proposed the following intended use statement:

> Epi proColon test is a qualitative *in vitro* diagnostic test for the detection of methylated Septin9 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 Septin9 DNA target.
>
> The test is indicated to screen patients for colorectal cancer who are defined as average risk for colorectal cancer (CRC) by current CRC screening guidelines. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. Men and women 50 to 85 years of age were included in Epi proColon clinical trial. Epi proColon test results, together with the physician's assessment of history, other risk factors, and professional guidelines, may be used to guide patient management.
>
> Epi proColon test is for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument.

1.1 Warnings

The Sponsor has proposed to include the following warnings in the product labeling:

- Epi proColon test is not intended to replace colorectal screening by colonoscopy.
- Epi proColon test is not intended to screen persons under the age 50 who are considered to be at average risk for colorectal cancer.
- Positive Epi proColon test results are not confirmatory evidence for the presence of colorectal cancer. 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 colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.
- Positive test results have been observed in clinically diagnosed patients with chronic gastritis, lung cancer and also in pregnant women.

1.2 Limitations

The Sponsor has proposed to include the following limitations in the product labeling:

- This product has been validated for the combination of Epi proColon Plasma Quick Kit (M5-02-001), Epi proColon Sensitive PCR Kit (M5-02-002), and Epi proColon Control Kit (M5-02-003) only. These kits and components (DNA extraction, bisulfite conversion or PCR kits) are not interchangeable or replaceable with other manufacturer's products.
- Epi proColon test has been validated for use only with plasma derived from blood collected with BD Vacutainer® K2 EDTA blood collection tubes (Becton Dickinson). Do not use this test with other clinical specimen types or with other blood collection tubes.
- Epi proColon test has been validated for use only with the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instruments with Sequence Detection Software v1.4 21 CFR Part 11 Module. Do not use with other instruments or software.
- Use of this test is limited to personnel experienced and trained in performing PCR assays. Good technique is essential and failure to follow instructions provided in these instructions may produce erroneous results.
- Detection of colorectal cancer is dependent on the amount of free circulating tumor DNA in the specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.
- Epi proColon test is an alternative screening method for patients who are defined as average risk for colorectal cancer by current screening guidelines, and who are unwilling, unable or do not undergo screening by other recommended screening methods.
- Epi proColon test has not been evaluated in persons:
  - Considered to be at higher risk for developing colorectal cancer, or with a previous history of colorectal polyps or colorectal cancer. Persons at higher risk include those with a family history of colorectal cancer, particularly with two or more first-degree relatives with colorectal cancer, or one or more first degree relative(s) less than 50 years of age with colorectal cancer.
  - With known hereditary non-polyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP).
  - With anorectal bleeding, hematochezia, or with known iron deficiency anemia.
- There is insufficient evidence to report programmatic sensitivity of Epi proColon test over an established period of time.
- 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.

- Epi proColon test demonstrated non-inferiority to a FIT test (OC FIT-CHEK® Polymedco), for sensitivity but not for specificity, indicating that Epi proColon test exhibited a higher rate of false positive results compared to the FIT test. See Performance Characteristics in IFU Section 13.
- Test results should be interpreted by a healthcare professional.

2 DEVICE DESCRIPTION

Epi proColon is used by prescription only. The device is an *in vitro* diagnostic assay for the qualitative detection of Septin9 gene methylation (in the promoter region of v2 transcript) in DNA isolated from human plasma.

2.1 Device Components

Epi proColon includes the following three product kits to be sold separately:

- Epi proColon Plasma Quick Kit;
- Epi proColon Sensitive PCR Kit;
- Epi proColon Control Kit.

The contents of Epi proColon Plasma Quick Kit are lysis binding buffer, magnetic beads, wash buffers, bisulfite solution, and protection buffer. Epi proColon Sensitive PCR Kit includes PCR mix and polymerase. Epi proColon Control Kit contains the negative and positive controls, which are to be included in each assay run.

BD Vacutainer® K2 EDTA blood collection tubes (cat. no. 366643) are required to perform Epi proColon test. They are not included in the kits, and must be purchased separately.

2.2 Principle of Operation

Blood (10 mL) is collected in BD Vacutainer® K2 EDTA tubes, and 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 according to the IFU.

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

Epi proColon Sensitive PCR Kit is used to amplify and detect the methylated Septin9 (mSEPT9) target region and a control region in the β-actin gene (ACTB) in one PCR reaction. The bisulfite-treated DNA is added 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 Epi proColon Control Kit. Both the positive and negative controls must meet the specified cutoff criteria for the run to be valid. Each sample 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 three 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 an ‘invalid’ result is reported.

2.3 Rationale for Device Design

Circulating cell-free nucleic acids in human blood were first reported in 1948 (Mandel P, 1948). Since then, the diagnostic potential of cell-free nucleic acids has been highlighted by a number of recent studies, demonstrating that plasma and serum samples from patients with various clinical pathological conditions – including cancer – contain cell-free DNA with related molecular alterations. Apoptosis or necrosis of the affected cells (e.g., cancer cells) may be a mechanism by which nucleic acids are released into the blood (Schwarzenbach H, 2011). Molecular alterations in the Septin9 gene (SEPT9) have been associated with ovarian (Russell SE, 2000), breast (Montagna C, 2003), prostate (Amir S, 2010), head and neck cancers (Bennett KL, 2008). Additionally, hypermethylation of SEPT9 has been detected in plasma of individuals with colorectal cancer (CRC) in the promoter region of the v2 transcript (Grützmann R, 2008). This region is generally unmethylated in samples from non-diseased subjects (Wasserkort R, 2013). Epi proColon test evaluates plasma samples derived from whole blood to detect the methylation status of CpG sites contained within the SEPT9 region with CRC-related hypermethylation.

3 BACKGROUND INFORMATION & REGULATORY HISTORY

3.1 Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer diagnosed in men and women in the United States. According to the American Cancer Society, in 2014, it is estimated that there will be 96,830 new cases of colon cancer and 40,000 new cases of rectal cancer diagnosed in the United States with approximately 50,310 patient deaths, making CRC the second leading cause of death from cancer among adults (www.cancer.org). CRC begins as a benign adenomatous polyp, which progresses to advanced adenoma (AA) with high-grade dysplasia and then to an invasive cancer (Markowitz SD, 2009). Invasive cancers that are confined within the colon wall (tumor-node-metastasis stages I and II) are curable. However, if untreated, the cancer may extend to regional lymph nodes (stage III) and then metastasize to distant sites (stage IV). Stage I and II tumors are treated by surgical excision, and up to 73% of stage III cases are treated by surgery combined with adjuvant chemotherapy. Although recent advances in chemotherapy have improved survival, stage IV disease is usually incurable. The risk of developing CRC increases with age and is greater in men than in women (www.cancer.org). Overall, the lifetime risk of developing CRC is about 5%.

3.2 Current Colorectal Cancer Screening Guidelines

There is strong evidence to suggest that screening for CRC reduces the incidence and mortality of the disease (CDC, 2013). Conventional screening for CRC includes both invasive and non-invasive options. Invasive tools include flexible sigmoidoscopy, double contrast barium enema, computed tomography colonography (CTC) and colonoscopy. Colonoscopy is considered the most accurate screening tool available. Non-invasive CRC screening tools include guaiac-based fecal occult blood testing (gFOBT) and immunochemical-based fecal occult blood testing (FIT). Patients who have a positive test result with any of these methods, except colonscopy, warrant further investigation with colonoscopy to confirm or discount the presence of CRC or polyps.

A number of professional societies and organizations have developed guidelines for CRC screening. Although the details of the recommendations differ, there is agreement that screening for average-risk persons should start at age 50 with repeat testing over time. Guidelines published in 2009 from the American College of Gastroenterology (ACG) recommend that colonoscopy conducted every 10 years remains the preferred CRC screening strategy (Rex DK, 2009). In settings where colonoscopy is not available due to economic limitations or eligible persons are not willing to undergo colonoscopy for screening purposes, the guidelines recommend that patients be offered an alternative screening test such as flexible sigmoidoscopy every 5-10 years, CTC every 5 years, or a cancer detection test such as FIT every year or fecal DNA testing every 3 years.

In March 2008, the American Cancer Society (ACS), the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology jointly recommended screening for CRC by the following: 1) high-sensitivity FOBT or FIT annually, 2) flexible sigmoidoscopy every 5 years, 3) double-contrast barium enema every 5 years, 4) CTC every 5 years, 5) colonoscopy every 10 years, or 6) fecal DNA testing at an unspecified interval (Levin B, 2008). In contrast, the US Preventative Services Task Force (USPSTF) does not recommend the use of fecal DNA testing and virtual colonoscopy. In October 2008, the USPSTF recommended screening for CRC using 1) FOBT annually, 2) sigmoidoscopy every 5 years with FOBT every 3 years, or 3) colonoscopy every 10 years (U.S. Preventive Services Task Force, 2008).

The guidelines also vary with respect to the approach to screening for CRC in patients 75 years of age and older. Recommendations from the ACG and ACS do not specify an age limit for CRC screening (Rex DK, 2009 and Levin B, 2008). The American Society for Gastrointestinal Endoscopy recommends that the decision to cease screening at a particular age should be considered between the patient and their physician on an individual basis considering the patient’s health status and prior screening history (www.asge.org). The USPSTF recommends against routine screening in adults 76-85 years of age, though there may be instances when considerations on an individual basis may support CRC screening (U.S. Preventive Services Task Force, 2008). The USPSTF further recommends that CRC screening not be conducted in patients over 85 years of age. Currently, the assessment and comparison of CRC screening methods in a testing program over time from a population perspective are limited to data from analytic modeling.

3.3 Colorectal Cancer Screening Sensitivity, Participation & Practice

Determination of test sensitivity from one-time use in a cross sectional study does not directly translate to screening program sensitivity, which is achieved through repeated testing over time (Imperiale TF, 2010). A test applied serially can have multiple opportunities to detect a lesion to the extent that results are independent at each use; however, if some lesions cannot be detected by a particular test (e.g., the lesion(s) does not and will not exhibit a particular molecular alteration), then results are not independent and cumulative sensitivity would not increase for those patients. Another factor that may affect screening program sensitivity is dwell times. For lesions growing quickly, a lower sensitivity test repeated more frequently may detect more disease compared with a higher sensitivity test performed less often (Ransohoff DF, 2013). Given that screening program sensitivity cannot rely on test sensitivity, there are randomized trials in progress comparing screening by FIT with colonoscopy (e.g., the Colonoscopy versus Fecal Immunochemical Test in Reducing Mortality from Colorectal Cancer [CONFIRM] trial, and the Colorectal Cancer Screening in Average-Risk Population: Immunochemical Fecal Occult Blood Testing versus Colonoscopy [COLONPREV] trial) (Levin TR, 2013).

In the United States, CRC screening participation has increased in recent years. According to the Centers for Disease Control and Prevention (CDC), CRC screening participation for persons 50 to 75 years of age increased from 54% in 2002 to 65% in 2010 (CDC, 2013). The CDC report cautions that data collected before 2011 cannot be compared with data from 2011 or beyond due to changes in the sample group responding to the survey. Taking into account the changes to the weighting of the data in 2011, the proportion of individuals undergoing CRC screening was 65.1% in 2012. Among this population, the most frequently used screening modality was colonoscopy (61.7%) followed by FOBT (10.4%) and then a combination of sigmoidoscopy and FOBT (0.7%). About one-third of the average risk population remains unscreened for CRC using a testing option recommended by USPSTF. Approaches such as organized population-based efforts, may be needed to facilitate progress in increasing CRC screening.

In clinical practice, the screening guidelines are not necessarily followed. One report found serious deviations from evidence-based recommendations in United States primary care (Nadel MR, 2010). Testing a single specimen collected during a digital rectal examination in the office is not an appropriate method for screening, but 24.9% of physicians reported using only this type of in-office tests, while another 52.9% reported using a combination of in-office and home tests. It was further reported that, instead of diagnostic colonoscopy as follow up for a positive test result, physicians recommend repeating the FOBT (17.8%) or using other tests (6.6%). These departures from CRC screening guidelines suggest that efforts are needed to inform physicians of appropriate test methods.

3.4 Regulatory Considerations

FDA is reviewing the *in vitro* diagnostic device Epi proColon under the premarket approval (PMA) process. To appropriately define test performance, it is important to evaluate the disease spectrum representative of the screening population. FDA has suggested that a cross-sectional clinical study supporting the performance of an *in vitro* diagnostic device for CRC screening be designed in the context of fecal immunochemical test (FIT) performance. FIT is a recommended screening modality across different guidelines. Fecal occult blood test (gFOBT) screening is supported by long-term longitudinal follow-up (Shaukat A, 2013). Additionally, since different studies have reported a range of FIT performance (Whitlock EP, 2008 and Lee JK, 2014), a direct head-to-head comparison to a FIT assay with well-documented CRC screening experience in the intended use setting is warranted to assess the performance of a new *in vitro* diagnostic device.

In the event that Epi proColon is approved, the practice of medicine with regard to CRC screening may change. Due to the caveats in extrapolating programmatic performance for CRC screening from cross sectional data, FDA has encouraged the Sponsor to propose a post-approval study (PAS) in order to facilitate Panel discussion regarding the need and approach for additional longitudinal performance data to adequately ensure safety and effectiveness of the device. In the absence of longitudinal performance results from a newly approved device, safety concerns surrounding program sensitivity may be mitigated by limiting product claims to one-time screening or consideration for follow-up screening by an independent method and reference to medical guidelines.

An additional consideration is the extent to which materials provided to patients and physicians properly inform decisions on screening and follow-up testing intervals and methods. In light of the trends in participation and deviations from screening recommendations in actual practice, FDA would like to ensure that materials provided to patients and physicians with *in vitro* diagnostic devices are appropriate within the context of current screening guidelines and the intended use. The Agency seeks feedback on whether safety and effectiveness of Epi proColon is adequately assured based on these considerations and Panel suggestions.

3.5 Marketing History

Epi proColon is a second generation Epi proColon test. The first generation test has been distributed commercially as a CE-marked test in Europe and the Middle East since 2009. The second generation Epi proColon test (Epi proColon® 2.0 CE) was launched in 2011 and is currently sold in the European Union and the Asian Pacific region. If the PMA is approved, Epi proColon will be commercially available in the United States.

4 NON-CLINICAL STUDIES

The Sponsor has conducted non-clinical studies to evaluate the analytical performance characteristics of Epi proColon. Brief descriptions of some of the studies are provided.

4.1 Analytical Sensitivity – Limit of Detection

Two studies were conducted to determine the limit of detection (LoD) of Epi proColon. Samples containing 0-50 pg/mL cell line DNA with mSEPT9 were repeatedly tested in both studies. One study evaluated nine levels of cell line DNA spiked into Tris buffer plus BSA. The LoD was 8.0 pg/mL (95% CI: 4.1, 15.5). The second study assessed the same nine levels of cell line DNA spiked into human plasma. The LoD was 4.7 pg/mL (95% CI: 2.5, 9.0). Logistic regression was used for analysis based on the qualitative test result. Samples without mSEPT9 were also evaluated, including cell line DNA spiked into Tris buffer plus BSA, Tris buffer plus BSA alone, and water. In all cases, negative results were observed.

4.2 Analytical Specificity

4.2.1 Cross Reactivity

The analytical specificity of Epi proColon was evaluated in several ways. First, electronic PCR and alignment analyses of the human genome demonstrated that only the mSEPT9 target sequence was predicted to amplify using Epi proColon assay. Second, probe specificity for the fully methylated mSEPT9 target sequence was empirically demonstrated using synthetic templates representing different combinations of cytosine methylation within the mSEPT9 probe region. Third, technical samples containing cell line DNA or sperm DNA that is unmethylated at SEPT9 were negative by Epi proColon.

The performance of Epi proColon was further evaluated in subjects taking common medications, subjects with chronic conditions, and subjects with non-CRC related cancers. For a subset of samples with a positive test result, the mSEPT9 target sequence was confirmed to be methylated, as determined by bisulfite sequencing. Study details are provided in the Appendix, Section 10.1.

4.2.2 Interference

The effects of ten potential interfering substances on the performance of Epi proColon were evaluated. Interference was not observed when the substances were tested at the following concentrations: albumin (26 mg/mL), bilirubin (0.2 mg/mL), cholesterol (5 mg/mL), glucose (10 mg/mL), hemoglobin (10 mg/mL), triglycerides (12 mg/mL), K_2EDTA (20 mg/mL), red blood cells (0.26% v/v), uric acid (0.235 mg/mL) and human sperm DNA (66 ng/mg). False positive results were detected when three substances were tested at higher concentrations: albumin (40 mg/mL), red blood cells (0.4% v/v) and human sperm DNA (100 ng/mL).

4.3 Reproducibility

Variability from different sources was assessed by testing 14 clinical sample pools at three sites with six operators (two per site) using three reagent lots and three PCR instruments. Plasma sample pools from CRC patients and healthy blood donors (self-declared) were tested. 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%). For reproducibility, the standard deviation and coefficient of variation ranges for mSEPT9 are 0.4 to 2.3 Ct and 1.4% to 6.0%, respectively. The corresponding ranges for ACTB are 0.2 to 0.4 Ct and 0.7% to 1.6%. Details are provided in the Appendix, Section 10.2.

4.4 Assay Cutoff

Epi proColon results are interpreted manually based on cycle threshold (Ct) values for mSEPT9 and ACTB. The thresholds were initially established using 156 specimens. Colonoscopy was used as the reference method and the PCR reactions were performed over 50 cycles. For mSEPT9, it was observed that mSEPT9 Cts greater than 40 were rare and none were above 45. For ACTB, the majority of Ct measurements were between 26 and 30, and few were close to 32. The Ct cutoffs for mSEPT9 and ACTB were established at 45 and 32.1, respectively.

A total of 203 subjects (203 CRC subjects and 100 with no evidence of disease, as determined by colonoscopy) were assessed to verify the assay cutoffs. The PCR reaction was performed over 50 cycles, and valid results were obtained from 197 specimens. Data analysis was performed using two sets of parameter settings. The distribution of the results confirmed the Ct cutoffs previously established; however, upon comparison of the two parameter settings, modifications were made to the settings for ACTB and a design change was implemented.

To verify the assay cutoffs with the modified analysis settings, 150 non-CRC samples previously collected in the PRESEPT study were evaluated (refer to Section 5 and Figure 1). Colonoscopy was used as the reference method and the PCR reactions included 50 cycles. Valid results were obtained for 149 samples and positive results were detected for mSEPT9 Ct values less than 45. All rounds of testing yielded consistent sensitivity and specificity results. Upon verification of the assay cutoffs, the PCR reaction for Epi proColon was set to 45 cycles.

4.5 Robustness

4.5.1 Failure Modes

To ensure that Epi proColon can detect major failure modes, 20 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 (Table 1). There was no effect on test results of the samples or run controls for 10 modes. The remaining modes were detected by invalid process controls and/or invalid internal ACTB control results.

Table 1. Modes tested in the Robustness Study

Mode	Process Step	Description
1	DNA extraction	Incorrectly thaw samples
2	DNA extraction	Incorrect sample volume
3	DNA extraction	Alcohol not added to reagent
4	DNA extraction	Incorrect alcohol concentration
5	DNA extraction	Add reagents in incorrect order
6	DNA extraction	Exchange reagents
7	PCR	Incorrect PCR analysis settings
8	DNA extraction	Incorrect bead binding time
9	DNA extraction	Incorrect magnetic rack
10	DNA extraction	Change instrumentation for binding step
11	DNA extraction	Inhomogeneous bead suspension
12	Bisulfite conversion	No mixing of reaction
13	Bisulfite conversion	Incorrect reaction time
14	DNA purification	Increase temperature for binding
15	DNA purification	Over-dry beads
16	DNA purification	No bead drying
17	PCR	Incorrectly store converted DNA
18	PCR	Incorrect DNA volume added
19	PCR	Incorrect storage of PCR mastermix
20	PCR	Incorrect storage of PCR reactions

4.5.2 Blood and Plasma Handling

The robustness of several parameters for blood handling, plasma preparation and storage were tested (Table 2). Blood samples from healthy donors were spiked with CRC plasma and tested alongside unspiked samples under various conditions. Compared to the standard process outlined in the IFU, there were no significant effects on test results of the samples or run controls for all tested conditions. Of note, the proportion of positive results for the unspiked samples (23%), across all test conditions, was comparable to the false positive rate observed in the clinical studies (see Sections 5 and 6). The pattern of positive test results in the unspiked samples did not appear to relate to a particular test condition.

Table 2. Conditions Tested for Blood and Plasma Storage and Processing

Mode	Process Step	Description
1	Standard process in IFU*	--
2	Blood storage	Extend storage to 6 hours at room temperature
3	Plasma storage	Store for 72 hours at 2-8°C, then at -80°C
4	Plasma storage	Store for 14 days at -25 to -15°C, then at -80°C
5	Plasma preparation	Single spin at 1100g for 10 min
6	Plasma preparation	Double spin at 1600 g for 14 min
7	Blood storage	Extend storage to 24 hours at 2-8°C

* Instructions for Use (IFU) states the following: plasma should be prepared within 4 hours after blood draw; plasma may be stored at 2-8°C for up to 72 hours, or at -25 to -15°C for up to 14 days. In addition, plasma is prepared with two centrifugation steps at 1350 g for 12 min each.

5 PIVOTAL CLINICAL STUDY

Two clinical studies were performed to demonstrate clinical performance of Epi proColon. The pivotal clinical study (VAL0018) was titled, “Pivotal Clinical Study for Epi proColon Test.” The study was designed to compare the performance of Epi proColon to that of colonoscopy using samples collected in a previous study (SPR006) titled “Prospective Evaluation of Septin9 Performance in CRC Screening” (PRESEPT). The study details are described in this Section. A supplemental clinical study (SPR0022) was conducted to compare the performance of Epi proColon and fecal immunochemical test (FIT) to colonoscopy (Section 6).

5.1 Study Objectives

5.1.1 Primary Objectives

The primary objective was detection of CRC by colonoscopy, followed by histological confirmation, compared to Epi proColon test result. Specifically, Epi proColon shall demonstrate sensitivity for CRC of 65% and specificity of 85% based on valid results for at least 95% of clinical samples and valid test runs in at least 90% of standard runs.[1]

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[1] The primary objectives did not include specified performance criteria based on statistical significance. Statistical significance means that the lower bound of the two-sided 95% confidence interval for the estimated performance measure should be above the target values (65% for sensitivity and 85% for specificity).

5.1.2 Secondary Objectives

Secondary objectives were to evaluate test positivity in advanced adenoma (AA), small polyps (SP), and specimens with no evidence of disease (NED).

5.2 Study Design

The pivotal study was designed to demonstrate the performance of Epi proColon compared to that of colonoscopy. 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 four clinical groups:

- Colorectal cancer (CRC) - Clinical/surgical diagnosis of invasive colorectal adenocarcinoma detected by optical colonoscopy and confirmed by histology for CRC cases (stages I-IV).
- Advanced adenomas (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.
- Small polyps (SP) – polyps < 10mm and without a villous component or HGD.
- No evidence of disease (NED) – no evidence of any of the above.

Plasma specimens evaluated in the pivotal study were previously collected under the PRESEPT study from June 2008 to January 2010. Enrollment in the PRESEPT study proceeded until 50 CRC subjects were enrolled. Eligible subjects who failed to meet the inclusion or exclusion criteria were withdrawn from the study. All study participants provided a blood sample prior to bowel preparation for colonoscopy. Whole blood was collected from each subject, processed to plasma, aliquoted and archived at -70°C. In 2011, randomized sample batches were shipped to one of three independent US laboratories for testing with Epi proColon for the pivotal study.[2]

5.3 Eligibility Criteria

The eligibility criteria are listed below for subjects to be included in the PRESEPT study, and for the archived specimens to be included in the pivotal study.

5.3.1 Inclusion Criteria

Subjects for PRESEPT

- Informed Consent provided;
- Capable of providing adequate health history;

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[2] Each participating laboratory underwent a training and qualification process prior to validation testing. For one operator at one of the sites, the parameter settings for analysis were adjusted for only one well to account for an irregular noise peak in a qualification run. The Epigenomics team reviewed the raw data and determined the adjustment to be acceptable. This protocol deviation resulted in qualification of the operator, who otherwise would not have qualified. Of note, the pivotal study was conducted before a design change to Epi proColon regarding review of irregular curves. The study was initially evaluated using revision 3 of the Instructions for Use (IFU); the results in this Executive Summary are based on revision 5 of the IFU. Based on either revision, however, the operator would not have qualified without the parameter adjustment.

- Age 50 or older at time of colonoscopy[3] (colorectal screening guideline-eligible[4]);
- Accessible for blood draw prior to start of bowel preparation for colonoscopy;
- First colonoscopy in lifetime.[5]

5.3.2 Exclusion Criteria

Subjects for PRESEPT

- 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 colorectal cancer (2 or more primary relatives with CRC; 1 or more primary relative(s) < 50 years with CRC; known HNPCC or FAP).

Specimens for Pivotal Study

- Gross hemolysis (bright orange or red color);
- Non-compliant with protocol for collection, processing, storage or shipping;
- Plasma samples with inadequate volume for Septin9 analysis.

5.4 Study Subjects

In the PRESEPT study, 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).

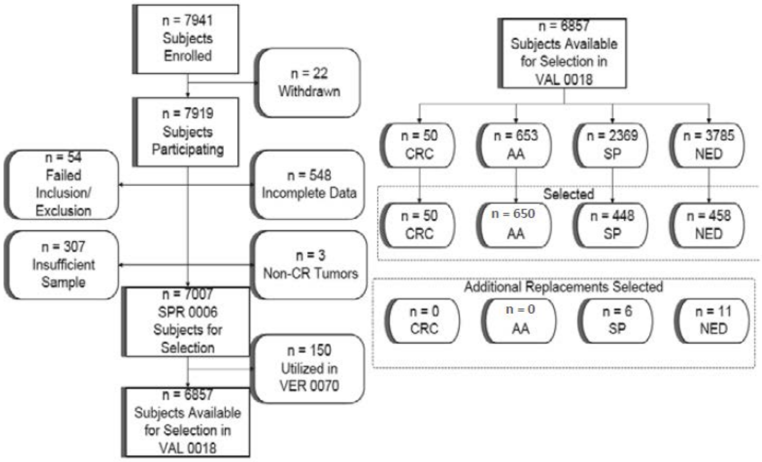

Figure 1. Subject Enrollment and Selection. Left: Subject enrollment, participation, exclusion[6], and selection for the PRESEPT study (SPR0006) are shown. Samples used in in the cutoff verification study (VER0070, described in Section 4.4) are also shown. Right: The flowchart on the right indicates the subjects selected for the pivotal clinical study (VAL0018).

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[3] Subjects enrolled in the study ranged from 49 to 92 years of age. One subject was 49 years old and had CRC; eight subjects were older than 85 years old and did not have CRC.

[4] The CRC screening guideline (2008) from the American Cancer Society, the US Multi-Society Task Force on colorectal cancer and the American College of Radiology was referenced in the PMA.

[5] In December 2008, the PRESEPT study was revised to include subjects having had flexible sigmoidoscopy greater than 5 years prior to the scheduled colonoscopy.

[6] Subjects with incomplete data include 516 subjects with colonoscopy not performed within time or incomplete (or inadequate) preparation and 32 subjects with no tissue.

Prior to the development of the current version of the device, a prospective evaluation of the Septin9 biomarker was conducted using the first generation commercially available assay. This evaluation was based on a subset of PRESEPT plasma samples that included all cancer patients and a subset of samples from the non-CRC clinical categories. Results from 53 CRC cases and from 1457 subjects without CRC yielded crude rates for sensitivity and specificity of 50.9% and 91.4%, respectively (Church TR, 2014).

In the pivotal study, a subset of archived specimens from 1623 subjects from the PRESEPT study was evaluated (Table 3). 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 4). The age range for all subjects was 49 to 92 years old. Epi proColon test results were valid for 1544 subjects. The reasons for subject exclusion due to invalid test results are listed in Table 5.

Table 3. Subjects selected for pivotal study

Clinical Group	PRESEPT	Pivotal Study	
	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.

Table 4. Demographic Distribution of Subjects with valid Epi proColon results

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.

5.5 Study Results

5.5.1 Primary Objectives

In total, valid Epi proColon results were obtained for 1544 of the 1623 samples evaluated (Table 3); 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) (Table 5). 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%), which meet the acceptance criterion that at least 90% of standard runs should be valid.

Table 5. Reasons for Subject Exclusion

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

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 6). The sensitivity of Epi proColon is 68.2% (95% CI: 53.4%, 80.0%). The point estimate falls within the pre-specified criterion that Epi proColon test shall demonstrate sensitivity for CRC of 65%. However, the 95% CI lower bound was 53.4%, which is below the targeted point estimate of 65%. In the non-CRC subgroups – AA, SP, and NED – the specificity is 78.8% (95% CI: 76.7%, 80.8%), which did not meet the criterion that Epi proColon shall demonstrate specificity of 85%.

Table 6. Pivotal Study 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 disease (CRC) and non-disease categories (AA, SP, NED) that was observed in the PRESEPT study (Table 7).

Point estimates and 95% CIs were computed by FDA based on bootstrap methodology. Furthermore, specificity can be weighted according to the proportions of non-CRC categories (AA, SP, NED) observed in the PRESEPT study. Given the positive detection rates observed in non-CRC groups, the weighted specificity is not expected to be different than the unadjusted specificity in the pivotal study.

Table 7. Adjusted Predictive Values

Parameter	Point Estimate (%)	95% CI
Positive Predictive Value	2.3	1.8, 2.9
Negative Predictive Value	99.7	99.6, 99.8
P(AA|negative)*	9.5	9.1, 9.9
P(SP|negative)†	35.2	33.8, 36.7
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

5.5.2 Secondary Objectives

In the CRC clinical group, the positive detection fraction (PDF) was further evaluated by tumor stage and tumor location (Table 8). PDFs across stage groups for CRC subjects are different with statistical significance (Fisher-Freeman-Halton test, 2-sided exact p-value = 0.026).

Table 8. Positive Detection Fraction by Tumor Stage and Location

Factor	Stratum	Epi proColon			
		Positive	Total	PDF (%)	95% CI
CRC Stage	Stage I	7	17	41	22, 64
	Stage II	10	12	83	55, 95
	Stage III	8	10	80	49, 94
	Stage IV	5	5	100	57, 100
Colon Location	Proximal	21	30	70	52, 83
	Distal	9	14	64	39, 84

The PDFs for each non-CRC subgroup range from 20% to 22% (Table 9). The differences between groups are not statistically significant (χ^2 test, 2 degrees of freedom, p-value = 0.76).

Table 9. Positive Detection Fraction in Non-CRC Groups

Non-CRC Group	Epi proColon			
	Positive	Total	PDF (%)	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

5.5.3 Demographic Variables

There is evidence of a decrease in specificity of Epi proColon with increasing age, as observed with the increasing false positive detection fractions (PDFs) with increasing age groups in non-CRC subjects (Table 10). PDFs for each age group for non-CRC subjects are different with statistical significance (Fisher-Freeman-Halton test, 2-sided exact p-value < 0.001). Similar analysis shows no evidence of significant differences in PDFs for CRC subjects across the same age categories (p-value = 1.0).

Table 10. Results by Diagnosis and Age

Age	CRC subjects				Non-CRC Subjects			
	Neg	**Pos**	**PDF (%)**	**95% CI**	**Neg**	**Pos**	**PDF (%)**	**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

Given that certain guidelines recommend routine screening for CRC up to 75 years of age, FDA conducted an additional analysis by grouping patients according to this age cutoff. The specificity in non-CRC patients who are 75 years old or younger is 79.5% (1116/1404) and the specificity in non-CRC subjects who are above 75 years of age is 68.8% (66/96). The difference in specificity between these groups is 10.7% (95%CI: 0.7%, 20.8%), which is statistically significant (p-value = 0.02).

For different ethnic categories, the PDFs for non-CRC subjects range from 18.1% to 27.1% (Table 11). Statistical analysis indicates that the PDFs for each category are not equivalent (Fisher-Freeman-Halton test, 2-sided exact p-value = 0.035). An increase in false positive detection fraction was observed in African-Americans, indicating that there is a decrease in specificity in African-Americans. Similar analysis for CRC subjects shows that there are no significant differences in PDFs across the same ethnic categories (p-value = 0.78).

Table 11. Results by Diagnosis and Ethnicity

Ethnicity	CRC subjects				Non-CRC Subjects			
	Neg	**Pos**	**PDF (%)**	**95% CI**	**Neg**	**Pos**	**PDF (%)**	**95% CI**
Other	1	1	50	2.6, 97.4	122	27	18.1	12.8, 25.1
Caucasian	12	27	69.2	53.6, 81.4	872	221	20.2	17.9, 22.7
African-American	1	2	66.7	20.8, 98.3	188	70	27.1	22.1, 32.9

Further analysis by diagnosis, age and ethnicity was conducted (Table 12). Four sub-categories are not represented among the CRC subjects. In non-CRC subjects who are Caucasian, the PDFs for different age categories range from 13.4% to 25.9%. These were different with statistical significance (Fisher-Freeman-Halton test, 2-sided exact p-value < 0.001), indicating that there is a decrease in specificity with increasing age among the Caucasians in the pivotal study.

Similar analyses show no evidence of significant differences in PDFs for Caucasian CRC subjects across age categories (p-value = 1.0), as well as for non-CRC subjects of “Other” ethnic origin across age categories (p-value = 0.51). Similarly, there are no significant differences in PDFs for African-American non-CRC subjects across age categories (p-value = 0.91).

Table 12. Results by Diagnosis, Age and Ethnicity

Ethnicity	Age	CRC subjects			Non-CRC Subjects		
		Neg	Pos	PDF (%)	Neg	Pos	PDF (%)
African-American	50-59	0	0	--	94	33	26.0
	60-69	1	2	66.7	64	25	28.1
	> 69	0	0	--	30	12	28.6
Caucasian	50-59	1	3	75.0	363	56	13.4
	60-69	6	13	68.4	314	97	23.6
	> 69	5	11	68.8	195	68	25.9
Other	50-59	0	0	--	54	11	16.9
	60-69	1	1	50.0	44	8	15.4
	> 69	0	0	--	24	8	25.0

Given that subjects were enrolled in the US and Germany, an additional analysis by site was conducted by FDA (Table 13). 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 13. Pivotal Study Results by Site

		Epi proColon					
		US			Germany		
		Negative	Positive	Total	Negative	Positive	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		57.7% (95% CI: 38.9%, 74.5%)			83.3% (95% CI: 60.8%, 94.2%)		
Specificity		78.6% (95% CI: 76.2%, 80.8%)			79.8% (95% CI: 74.7%, 84.1%)		

5.6 Study Conclusions

The pivotal clinical study was designed to examine patients of average risk who would participate in screening by colonoscopy. The study is therefore not applicable for use of Epi proColon as a substitute for colonoscopy in settings of heightened clinical concern, including high risk patients (e.g., predisposition due to genetics or gastrointestinal disease), diagnostic colonoscopy (e.g., patients with signs or symptoms), or surveillance colonoscopy (e.g., patients with personal history of colon cancer or polyps). The impact of device use in patients who would not participate in screening colonoscopy cannot be determined from this study.

The pivotal clinical study does not meet all of the pre-specified primary performance objectives. The sensitivity of Epi proColon for CRC was 68.2%. The point estimate falls within the pre-specified criterion that Epi proColon test shall demonstrate sensitivity for CRC of 65%. However, the 95% CI lower bound was 53.4% and is below the targeted point estimate of 65%. In the non-CRC group (comprised of AA, SP, and NED), the specificity is 78.8% (95% CI: 76.7%, 80.8%). This does not meet the criterion that Epi proColon shall demonstrate specificity of 85%. The false positive rate of 21% is higher than expected, indicating that more individuals (than expected) will be recommended to undergo a follow-up diagnostic procedure, such as colonoscopy, which will be negative.

Age and ethnicity significantly affect the PDF in non-CRC subjects. No evidence was found that age and ethnicity impact the PDF in CRC subjects. For the Caucasian non-CRC population the Epi proColon positivity rate increases with age. The increase in false positive detection fractions associated with increasing age is consistent with published reports demonstrating that there are age-related increases in methylation (Ahuja N, 2000 and Xu Z, 2014). An increase in false positive detection fraction was also observed in non-CRC African Americans. Of note, 53% of subjects in this ethnic group were enrolled at one clinical site; however, the higher positivity rate may be consistent with reports of higher CRC incidence in African-Americans, who are generally recommended to begin CRC screening at age 45, rather than 50 (Rex DK, 2009).

FDA Commentary: Since a cross sectional study addresses test sensitivity, but not screening program sensitivity, FDA would like the Panel to discuss issues of Epi proColon performance for one-time screening separately from repeated use. In the pivotal study, sensitivity for CRC met the pre-specified acceptance criterion based on the point estimate, but it was not statistically significant. Specificity did not meet the acceptance criterion. The reasons for the relatively low specificity estimate are unclear; however, there are several possibilities. First, some Epi proColon false positives could represent lesions missed at colonoscopy. Consistent with this, results from the analytical studies indicate that, in certain instances, positive Epi proColon results from non-CRC patients are true amplification signals that originate from methylated Septin9 target sequence in the plasma, as evidenced by bisulfite sequencing. Second, the false positive results could result from the presence of cancer other than CRC. In the analytical specificity study assessing cross reactivity of Epi proColon, positive test results were detected in several non-CRC cancer specimens, including hepatocellular carcinoma, as well as cancers of the stomach, kidney, lung, bladder, prostate, and breast (Appendix, Section 10.1). Third, the false positive results could reflect the presence of extremely low non-pathological levels of Septin9. Results from the reproducibility study with three specimen pools from healthy NED subjects indicate that the agreement with the expected test result in replicate testing was 75% (95% CI: 59%, 86%) relative to the clinical status (Appendix, Section 10.2). Thus, repeated testing of a non-CRC specimen does not consistently yield a negative result. The lower specificity leads to the potential for an increase in avoidable negative diagnostic colonoscopies and colonoscopy-related adverse events.

The pivotal study does not address the extent to which Epi proColon may influence initial CRC screening test participation, adherence to follow-up diagnostic colonoscopy, and diagnostic yield. FDA seeks Panel input on whether this device is safe and effective as a one-time screening test in light of the observed performance for sensitivity and specificity, the comparable positivity rates in AA and NED subgroups, and the fact that the study was cross sectional, and not programmatic. Additional considerations for discussion are alternative intended uses, such as an adjunctive second-line option after FIT, prominent labeling cautions highlighting relative Epi proColon performance compared to other screening options (including colonoscopy and FIT), or other Panel suggestions.

FDA also requests Panel feedback regarding the consistency of device performance, considering that analyses should be interpreted with the caution that the clinical studies were not designed to assess test performance in subgroups. In particular, the observation that specificity decreased with advancing subject age may be considered in view of the published literature demonstrating that epigenetic changes may accumulate with age, as well as the upper age limit considerations in clinical guidelines. The Agency seeks Panel input on whether this finding merits mention in the product labeling, including materials for patients and physicians.

6 SUPPLEMENTAL CLINICAL STUDY

This study was titled, “Comparison of the Performance of Epi proColon and Fecal Immunochemical Test, post Colonoscopy in Subjects with Colorectal Cancer and Pre-Colonoscopy in Subjects from a Guideline-Eligible Screening Population.” Matched blood and stool samples were collected prospectively to compare the performance of Epi proColon and a commercially available fecal immunochemical test (FIT) to colonoscopy results.

6.1 Primary Study Objectives

The primary objective was to demonstrate non-inferiority in the clinical performance of Epi proColon to a commercially available FIT assay. Non-inferiority of Epi proColon in CRC subjects is demonstrated if the one-sided 95% confidence interval (CI) for the difference of sensitivities of Epi proColon and FIT is below the non-inferiority margin of 0.1. Also, the performance of Epi proColon is considered non-inferior to FIT in NED subjects if the one-sided 95% CI for the difference of specificities of Epi proColon and FIT is below the non-inferiority margin of 0.2.[7, 8]

6.2 Study Design

The study was designed to compare the performance of Epi proColon and a commercially available Fecal Immunochemical Test (FIT) 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 the pivotal clinical study, subjects were classified into four clinical groups based on colonoscopy results: CRC, AA, SP and NED subjects. Subjects were then enrolled into the following two study arms:

- Group A - Patients have invasive colorectal cancer (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 up to have targeted quotas of 100 and 200 subjects, respectively, in order to estimate sensitivity and specificity. Based on the Sponsor’s assumptions that FIT

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[7] According to the clinical protocol, the primary criterion for specificity was pre-specified for NED subjects. However, the Sponsor provided all specificity analyses and results based on all non-CRC subjects, including NED, SP and AA patients.

[8] One-sided 95% confidence intervals (CIs) were pre-specified in the study objectives. Two-sided 95% CIs are presented in this Executive Summary, which were provided by the Sponsor at FDA’s request.

specificity is 90% and Epi proColon specificity is 84%, the study would have 99% power with 200 non-CRC subjects to meet the non-inferiority margin of 20%. For Group A sample size estimation, the sensitivities for FIT and Epi proColon are assumed at 80%. The study would have 54% power with 100 CRC subjects to meet the non-inferiority margin of 10%. 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. Results from each test were compared to colonoscopy results. Data monitoring at the central testing lab was performed by Epigenomics and monitoring at the clinical sites was performed by PRA.

6.3 Eligibility Criteria

6.3.1 Inclusion Criteria

Group A

- 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 colorectal carcinoma (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.

Group B

- 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.

6.3.2 Exclusion Criteria

Group A Only:

- Subjects with curative biopsy during colonoscopy.

Groups A and B:

- 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.

6.4 Study Subjects

A total of 337 subjects were enrolled prospectively from 61 US sites. Thirty-six subjects did not meet the inclusion/exclusion criteria (Table 14), resulting in 301 remaining subjects.

Table 14. Subjects Excluded from Supplemental Clinical Study

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

Of the remaining 301 subjects, all provided plasma samples and 290 provided stool samples that were evaluable (Table 15). 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.

Subjects ranged from 50 to 85 years of age; one subject was 85 years old and had CRC, as detected by colonoscopy. 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, colorectal cancer. 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. Demographic information is summarized in Table 16.

Table 15. Supplemental Clinical Study Samples

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

Table 16. Demographic Distribution of Subjects

Factor	Stratum	Group A	Group B
Gender	Female	32%	62%
	Male	68%	38%
Age	50-59	24%	64%
	60-69	37%	25%
	> 69	39%	11%
Ethnicity	Caucasian	69%	70%
	African-American	11%	14%
	Hispanic	17%	12%
	Other	4%	4%

6.5 Study Results

6.5.1 Primary Objectives

Based on the reference standard of colonoscopy, the sensitivity and specificity for CRC of Epi proColon were determined for all 301 subjects with plasma samples (Table 17), as well as for the subset of 290 subjects with matched blood and stool samples (Table 18). The sensitivities for both groups were similar at 73.3% and 72.2%, respectively.

Table 17. Results for Epi proColon and Colonoscopy on All Samples

		Epi proColon		
		Positive	**Negative**	**Total**
Colonoscopy	**CRC**	74	27	101
	Non-CRC	37	163	200
	Total	111	190	301
	Sensitivity	73.3% (95% CI: 63.9%, 80.9%)		
	Specificity	81.5% (95% CI: 75.5%, 86.3%)		

Table 18. Results for Epi proColon and Colonoscopy on Matched Samples

		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%)		

The sensitivity and specificity for CRC with FIT, relative to colonoscopy, were also determined (Table 19). The difference in sensitivity between FIT and Epi proColon is -4.2% (95% CI: -16.2%, 8.1%), in favor of Epi proColon. This meets the pre-specified non-inferiority margin of 10%. The difference in specificity is 16.6% (95% CI: 10.6%, 22.9%), in favor of the FIT test. This does not meet the pre-specified non-inferiority margin of 20%.

Table 19. Results for FIT and Colonoscopy on Matched Samples

		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%)		

Assuming CRC prevalence of 0.7%, as observed in the PRESEPT study, diagnostic likelihood ratios (DLRs) and predictive values (PVs) were determined (Table 20). The relationship between the positive DLRs reveals an advantage for FIT, which reflects the difference in specificity estimates between the two methods. In contrast, the negative DLRs are equivalent, as are the negative PVs.

Table 20. Diagnostic Likelihood Ratios (DLRs) and Predictive Values (PVs)

Parameter	**Epi proColon**		**FIT**	
	Point Est	**95% CI**	**Point Est**	**95% CI**
Positive DLR*	3.96	2.89, 5.42	26.26	10.94, 63.05
Negative DLR†	0.33	0.24, 0.46	0.33	0.25, 0.44
Positive PV	2.72%	2.00%, 3.68%	15.62%	7.16%, 30.77%
Negative PV	99.77%	99.68%, 99.83%	99.77%	99.69%, 99.83%

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

6.5.2 **Additional Analyses**

The results from matched specimens for CRC subjects (Groups A and B) suggest that there is overlap in the CRC subjects detected by Epi proColon and FIT (50/97) (Table 21). There are also non-overlapping results whereby a subject was positive by only one of the tests. When considering the “believe the positive” combination of Epi proColon and FIT results, the sensitivity in the CRC cohort is 88.7% and the specificity is about 78.8% (Table 23). For this combination scenario, the positive and negative diagnostic likelihood ratios (DLRs) are 4.17 and 0.14, respectively. An increase in positive DLR and a decrease in negative DLR for the combination with respect to Epi proColon imply that positive and negative predictive values, respectively, are greater for the combination than for Epi proColon alone for the same prevalence of cancer.[9]

Table 21. Results for FIT vs. Epi proColon on Matched CRC Samples

		Epi proColon		
		Positive	**Negative**	**Total**
FIT	**Positive**	50	16	66
	Negative	20	11	31
	Total	70	27	97

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[9] The difference in diagnostic likelihood ratios between the combination (Epi proColon and FIT) and Epi proColon have not been verified for statistical significance.

Table 22. Results for FIT vs. Epi proColon on Matched Non-CRC Samples

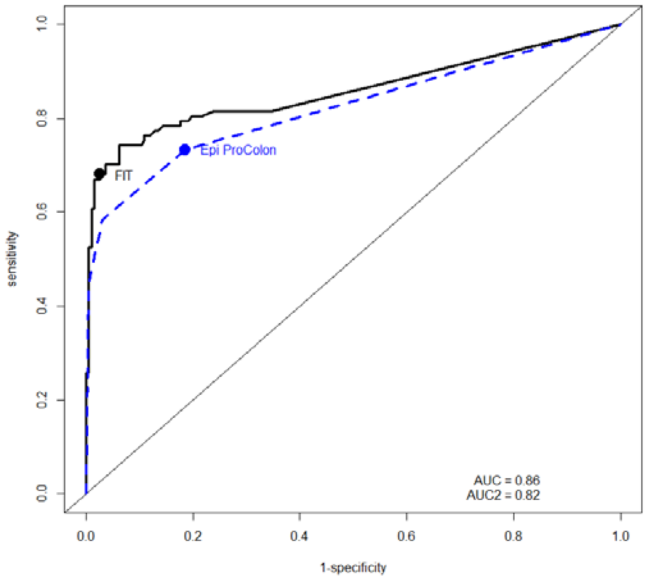
		Epi proColon		
		Positive	**Negative**	**Total**
FIT	**Positive**	1	4	5
	Negative	36	152	188
	Total	37	156	193

Table 23. Results for FIT or Epi proColon vs. Colonoscopy on All Matched Samples

		FIT or Epi proColon		
		Positive	**Negative**	**Total**
Colonoscopy	**CRC**	86	11	97
	Non-CRC	41	152	193
	Total	127	163	290
	Sensitivity	88.7% (95% CI: 80.8%, 93.5%)		
	Specificity	78.8% (95% CI: 72.4%, 83.9%)		

In addition, 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 by FDA (Figure 2). 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). ROC curves depict the entire set of operating characteristics (false positive fraction, true positive fraction) possible with each test, which allows for comparison of the tests at any operating point. The two tests can also be compared in terms of the area under the ROC curve (AUC), a global summary that may be interpreted as the average value of sensitivity for all possible values of specificity, or the average value of specificity across all possible values of sensitivity. The AUC for FIT is greater than the one for Epi proColon (0.86 vs. 0.82). This comparison has not been assessed for statistical significance.

Figure 2. ROC curves for sensitivity vs. false positive fraction (1-specificity) for FIT (black solid line) and for Epi proColon (blue dashed line) over all positivity thresholds. The sensitivity vs. false positive fraction (1-specificity) based on the pre-specified cutoffs for FIT (black dot) and Epi proColon (blue dot) are also shown.



Diagnostic performance comparison of the two tests was conducted on matched samples (n=290), as described in Section 6.5.1. Additional analyses to account for the 11 subjects with missing FIT results may be conducted by assuming the data are missing at random and imputing the missing FIT results. Such analyses had no effect on the study results. That is, non-inferiority of Epi proColon to FIT was met for sensitivity, but not for specificity.

6.5.3 Demographic Variables

Subgroup analyses were conducted to determine whether the performance of Epi proColon is affected by various demographic factors, such as tumor characteristics, age and ethnicity. Test positivity was observed for all tumor stages (Table 24). Test positivity in each clinical subgroup included in the non-CRC group is also provided in Table 25.

Table 24. Positive Detection Fraction by Tumor Characteristics

Factor	Strata	Epi proColon		FIT	
		Point Est	95% CI	Point Est	95% CI
Tumor Stage	Stage 0	100% (2/2)	34.2, 100	0% (0/2)	0, 65.8
	Stage I	61.5% (16/26)	42.5, 77.6	65.4% (17/26)	46.2, 80.6
	Stage II	80.0% (16/20)	58.4, 91.9	80.0% (16/20)	58.4, 91.9
	Stage III	65.2% (15/23)	44.9, 81.2	82.6% (19/23)	62.9, 93.0
	Stage IV	92.3% (12/13)	66.7, 99.6	58.3% (7/12)	32.0, 80.7
	Unknown*	76.5% (13/17)	52.7, 90.4	50.0% (7/14)	26.8, 73.2

* This includes subjects who did not undergo surgery within the time frame of the study, subjects who elected first-line treatment other than surgery, died during the study, and had pathology on biopsy alone.

Table 25. Positive Detection Fraction in Non-CRC Groups

Non-CRC Group	Epi proColon		FIT	
	Point Est	95% CI	Point Est	95% CI
AA	13.8% (4/29)	6, 31	7.4% (2/27)	2, 23
SP	14.3% (11/77)	8, 24	1.3% (1/75)	0.1, 7
NED	23.4% (22/94)	16, 33	2.2% (2/91)	0.6, 8
Total	18.5% (37/200)	14, 25	2.6% (5/193)	1, 6

There was no evidence for increasing test positivity based on gender or ethnicity for Epi proColon (Table 26). There was also no trend with increasing age, although test positivity with FIT seemed to increase with age. Although not significant, Epi proColon detected slightly more CRC cases in females, and there was a slight increase in test positivity in African-Americans.

Table 26. Positive Detection Fraction by Age and Ethnicity in CRC subjects

Factor	Strata	Epi proColon		FIT	
		Point Est	95% CI	Point Est	95% CI
Gender	Female	78.8% (26/33)	62.2, 89.3	63.3% (19/30)	45.5, 78.1
	Male	70.6% (48/68)	58.9, 80.1	70.1% (47/67)	58.3, 76.5
Ethnicity	Caucasian	71.4% (50/70)	60.0, 80.7	73.5% (50/68)	62.0, 82.6
	African-American	90.0% (9/10)	59.6, 99.5	70.0% (7/10)	39.7, 89.2
	Hispanic	70.6% (12/17)	46.9, 86.7	46.7% (7/15)	24.8, 69.9
	Other	75.0% (3/4)	30.1, 98.7	50.0% (2/4)	15.0, 85.0

6.6 Study Conclusions

In the supplemental clinical study, the observed sensitivity for Epi proColon is 72.2% (95% CI: 62.5%, 80.1%) and the specificity is 80.8% (95% CI: 74.7%, 85.8%) for 290 plasma samples. Testing of 290 stool samples with the commercially available FIT test yielded a sensitivity and specificity of 68.0% (95% CI: 58.2%, 76.5%) and 97.4% (94.1%, 98.9%), respectively. Of note, Group A sampling occurred after colonoscopy and subjects with curative biopsy were excluded from the study, which do not reflect the intended use setting. Despite the departure from the intended use, the performance of Epi proColon in this study is similar to the results from the pivotal study, i.e., sensitivity of 68.2% and specificity of 78.8%.

With respect to non-inferiority margins between Epi proColon and FIT, this study met the pre-specified success criteria for sensitivity, but not for specificity. The difference in sensitivity is -4.1% (95% CI: -16.2%, 8.1%). The 95% CI upper bound is 8.1%, which is below the pre-specified non-inferiority margin of 10%. In contrast, the pre-specified non-inferiority margin for specificity of 20% was not met. The difference in specificity between the two test methods is 16.6% (95% CI: 10.6%, 22.9%). The 95% CI upper bound of 22.9% is above 20%. Compared to Epi proColon, FIT results in fewer false positive patients referred to colonoscopy after a positive result in order to identify about the same number of CRC patients.

Based on the results obtained for sensitivity, both Epi proColon and FIT tests yield positive results at a similar rate in the CRC population. When the test results from both assays are considered in a “believe the positive” combination (i.e., a positive result is declared if either one or both tests are positive), the sensitivity increases to 88.7%, as compared to 72.2% for Epi proColon and 68.0% for FIT. Specificity decreases in this combined scenario to about 78.8%, as compared to 80.8% for Epi proColon and 97.4% for FIT. Similarly, the positive DLR increases from 3.96 for Epi proColon to 4.17 for the combination, implying that the positive predictive value is greater with the combination than with Epi proColon alone given the same prevalence of CRC. There was also a decrease in the negative DLR from 0.33 for Epi proColon to 0.14 for the combination, implying that the negative predictive value is greater with the combination than with Epi proColon alone, assuming the same cancer prevalence. The differences in the diagnostic likelihood ratios have not been assessed for statistical significance.

FDA Commentary: In addition to the cautions for study interpretation discussed in Section 5.7 for the pivotal clinical study, this head-to-head device comparison with FIT has additional methodological caveats. For example, specimen collection for CRC participants (Group A) occurred post-colonoscopy, which is inconsistent with the intended use setting, and spectrum bias may have been introduced by therapeutic colonoscopy for earlier disease. Also, while the study met the goal for sensitivity, the study did not meet the specificity goal of non-inferiority with a margin of 20%. Given that the observed FIT specificity was larger than expected and the observed specificity for Epi proColon was smaller than expected, this study achieved a power of 28% based on the observed specificity for FIT and Epi proColon (97.4% and 80.8%, respectively). This study is consistent with decreased specificity of Epi proColon observed in the pivotal study. Additionally, there is no statistically significant sensitivity benefit of Epi proColon relative to FIT. Thus, there is uncertainty as to whether Epi proColon is an appropriate substitute for CRC screening options such as FIT. In light of these considerations, FDA seeks

Panel input on whether the Epi proColon is safe and effective as a one-time screening test comparable to FIT.

As compared to the FIT test used in the study, the lower specificity for Epi proColon leads to the potential for a relative increase in avoidable colonoscopies and the adverse events associated with colonoscopies (Appendix, Section 10.3). To demonstrate the potential benefits and risks, the diagnostic yield from both tests in a population of 100,000 subjects was projected (Appendix, Section 10.3.1). Under the assumption that CRC prevalence is 0.7%, as observed in the PRESEPT study, 700 subjects (among 100,000) are expected to have CRC. Prevalence is then applied to the sensitivity for CRC and specificity for non-CRC observed in the study to project the numbers of true positives and false negatives for each test. In total, Epi proColon is expected to detect 29 more CRC subjects than FIT, as well as 16,464 additional false positive non-CRC subjects. The risk of an adverse event resulting from an avoidable follow-up colonoscopy can also be projected (Appendix, Section 10.3.2). Assuming the risk is 0.68% (Rutter CM, 2012), 112 additional serious adverse events (e.g., perforation, hemorrhage, or acute diverticulitis) from a colonoscopy can be expected after a false positive Epi proColon test, as compared to FIT. FDA seeks Panel input on whether the benefits outweigh the risks for use of Epi proColon.

One of the Sponsor’s proposed limitations for Epi proColon is as an alternative screening method for patients who are defined as average risk for CRC by current screening guidelines, and who are unwilling, unable or do not undergo screening by other recommended screening methods (Section 1.2). The pivotal and supplemental studies do not assess Epi proColon performance in this patient population. Like other screening tests, it is uncertain how offering Epi proColon to those who are unwilling, unable or do not undergo screening by other recommended screening methods would compare to other approaches, such as organized population-based efforts, to increase CRC screening participation. Use of any screening test, including colonoscopy, requires sufficient awareness and counseling regarding the test’s benefits and risks and appropriate use. Epi proColon, or any other screening test, may not be an appropriate alternative if patients do not undergo screening by other recommended methods due, in part, to insufficient awareness and counseling. The importance of this concern may not be apparent to patients and physicians if positioned in the context of multiple proposed limitations. The Agency requests Panel feedback regarding these issues.

Since the Sponsor has not provided longitudinal performance for their device, there is uncertainty regarding optimal follow-up of a negative result. The frequency and method of follow-up testing can influence screening program sensitivity due to the degree of test independence and dwell times. Given that Epi proColon performance was compared to FIT, it may be appropriate for discussion of follow-up screening in the context of the FIT screening interval. The lack of data regarding device performance in patients previously testing negative may prompt a preference for testing by an independent approach. FDA seeks Panel input regarding the adequacy of the Sponsor’s proposed warning that patients with a negative Epi proColon test result should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

7 POST-APPROVAL STUDY

Note: The inclusion of a Post-Approval Study section in this summary should not be interpreted to mean that FDA has made a decision or is making a recommendation on the approvability of this PMA device. The presence of a post-approval study plan or commitment does not in any way alter the requirements for premarket approval and a recommendation from the Panel on whether the risks outweigh the benefits. The premarket data must reach the threshold for providing reasonable assurance of safety and effectiveness before the device can be found approvable and any post-approval study could be considered. The issues noted below are FDA's comments regarding potential post-approval studies, for the Panel to include in the deliberations, should FDA find the device approvable based upon the clinical premarket data.

The FDA review team is seeking input on whether or not a post-approval study (PAS) should be required as a condition of approval, if Epi proColon is approved. Through review of the premarket data, FDA has identified potential postmarket concerns, which could be addressed through a PAS conducted to evaluate programmatic performance of the device in relationship to screening interval including the following:

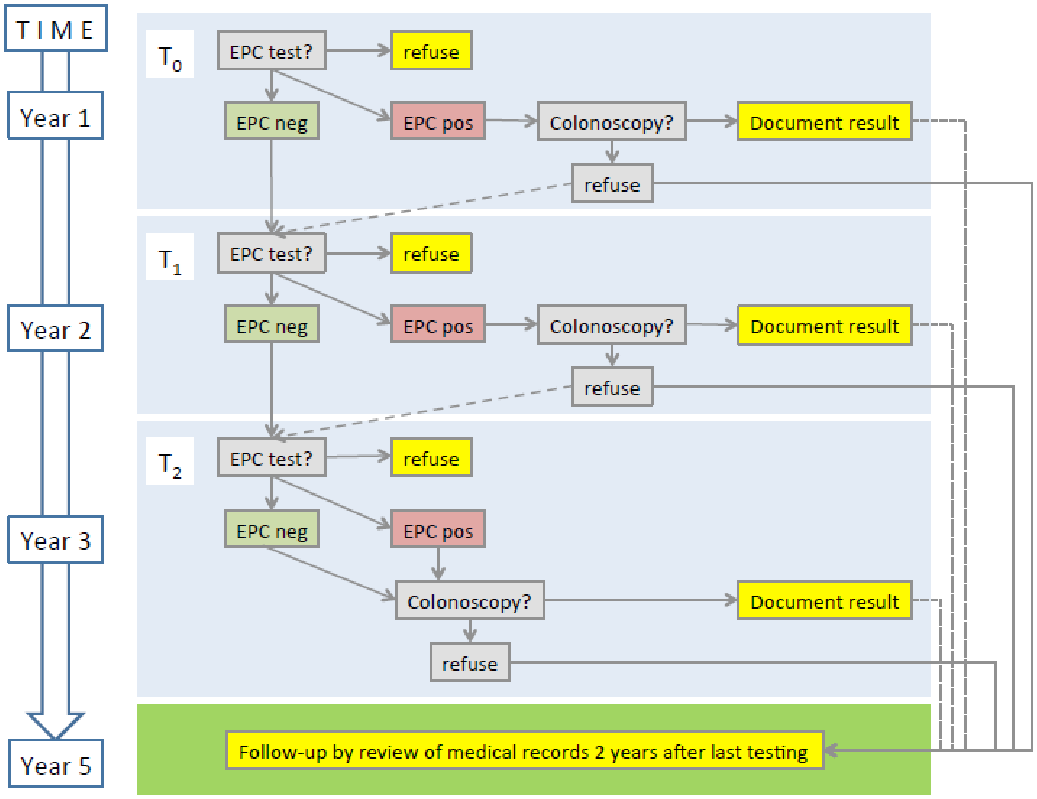
- Negative to positive conversion rate;
- Diagnostic yield;
- Predictive values.

FDA and the Sponsor have begun to work together to design this potential study. An overview of the proposed PAS protocol is provided in the following section.

7.1 Overview of Proposed Post- Approval Study

The Sponsor is proposing a study aimed at determining the programmatic performance of Epi proColon when testing is carried out annually for 3 years (Figure 3). Briefly, if subjects test positive by Epi proColon, they will be referred for diagnostic colonoscopy. If subjects test negative by Epi proColon, they will be tested one year later for up to three years. Subjects who test negative for all three years will be recommended to undergo colonoscopy. For all subjects enrolled in the study - both test positives and negatives - CRC-related medical records will be reviewed 2 years after the last Epi proColon test result.

Figure 3. Post-Approval Study Scheme



7.1.1 Study Population

The study population will reflect the intended use population for the device, such that the following criteria are met:

- Average risk population, according to the USPSTF recommendations for CRC screening;
- Representation of each gender, different age groups, and different ethnic backgrounds;
- No previous history of screening for CRC by colonoscopy;
- Subjects recruited from clinical sites utilizing Epi proColon.

7.1.2 Study Objectives

The programmatic performance of annual testing with Epi proColon will be evaluated based on:

- Diagnostic yield – per year and cumulative;
- Test positivity and positive predictive value – per year and cumulative;
- Negative predictive value – cumulative.

The study will also assess the compliance rate for screening with Epi proColon over a three-year period, and determine the rate of adherence to follow-up colonoscopy after a positive Epi proColon result.

7.1.3 Study Hypothesis

An annual screening program with Epi proColon significantly lowers the probability of carrying undetected CRC, such that NPV3 (i.e., the probability of not having CRC in individuals who test negative with annual Epi proColon testing for three years) > 1 - CRC prevalence, with statistical significance of $\alpha = 0.05$.

7.2 Post-Approval Study Considerations

Current CRC screening guidelines recommend that patients undergo routine screening with repeat testing over time. Within the context of these guidelines, the performance of Epi proColon has not been evaluated for repeat testing. The premarket data was solely based on cross-sectional studies in which specimens were tested at one time point. Thus, a post-approval study (PAS) intended to evaluate the longitudinal performance of Epi proColon in the postmarket setting may support long-term safety and effectiveness.

The design of a PAS may depend upon the manner in which Epi proColon is intended to be used as a screening method for CRC. For example, if Epi proColon is intended as a comparable alternative to accepted screening modalities, such as FIT, then establishing the performance of Epi proColon in relation to annual FIT may be appropriate. It may also be important to have study safeguards in the event that Epi proColon programmatic performance for repeat testing is inferior to FIT. Offering FIT testing could provide greater safety for study participants, but could also complicate understanding of the performance of Epi proColon repeat testing when used alone.

If a PAS is recommended as a condition of PMA approval, there are further considerations to be addressed regarding the study design. First, the appropriate study population criteria may depend on the intended use of the device. Second, not all study objectives, such as screening compliance rate with Epi proColon and adherence rate to follow-up colonoscopy, are associated with study hypotheses. Third, the appropriateness of the proposed study hypothesis to support annual testing with Epi proColon is unclear. It is possible that NPV3 for Epi proColon could be significantly lower than that for FIT even though both values may exceed (1-CRC prevalence). Such a result may suggest that Epi proColon is not comparable to FIT as an annual screening method. It is also possible that NPV3 for Epi proColon could be largely attributed to testing at the first time point, T0, with minimal contribution from the subsequent two time points, T1 and T2. A further possibility is that a device that works at random (sensitivity + specificity = 100%) could achieve the study hypothesis.

For discussion of the proposed longitudinal study, the Agency would appreciate advisory committee feedback concerning key issues, such as whether the study population is appropriate, whether the proposed investigation demonstrates statistically and clinically meaningful performance for repeat testing at an appropriate interval over an adequate follow-up period, whether allowing study participants to forgo annual FIT testing is appropriate based on current evidence, and other issues identified by the Panel.

8 PANEL QUESTIONS

8.1 Questions for Panel Discussion

1. In the pivotal trial, Epi proColon has a sensitivity of 68% (95% CI: 53%, 80%) and a specificity of 79% (95% CI: 77%, 81%). In the FIT comparison study to assess non-inferiority of Epi proColon, the goal for sensitivity was met, but the goal for specificity was not achieved. The decreased specificity of Epi proColon was not associated with a clear benefit in sensitivity when compared to a commercially available FIT test. The lower specificity could lead to an increase in the number of avoidable colonoscopies. While colonoscopies are considered the standard of care and recommended in CRC screening guidelines, there are adverse events associated with such invasive procedures. In the non-clinical studies, non-CRC specimens are not consistently detected by Epi proColon. In addition, there are some other cancer types for which methylated Septin9 is detected by Epi proColon.

    a. Do these outcomes adequately demonstrate effectiveness of Epi proColon within the context of the proposed intended use and current recommendations for colorectal cancer screening?[10]

    b. If yes, do the data support screening with Epi proColon as (i) a second-line option only in patients declining FIT, (ii) an alternative for FIT, (iii) other option?

    c. Based on the results of the pivotal and supplemental clinical studies, do the data allow for adequate assessment of the benefits versus risks of Epi proColon?[11]

2. In the pivotal study, Epi proColon results in non-CRC subjects were affected by demographic factors, such as age and ethnicity. In addition to the proposed age limitation (i.e., 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), does the current data warrant one of the following with respect to certain patient subgroups:

    a. Additional labeling considerations (e.g., warning, limitation) for patients who are above 75 years of age and/or African Americans?

    b. Precaution about potential for increased false positive rate in patients who are above 75 years of age and/or African Americans?

3. The proposed claims do not rule out repeat testing as part of the CRC screening program with Epi proColon. Cross-sectional performance at one time point was established in the pivotal and supplemental clinical studies. Follow-up longitudinal performance data on patients that tested negative with Epi proColon were not provided. The Sponsor has suggested a limitation (i.e., There is insufficient evidence to report programmatic sensitivity of Epi proColon test over an established period of time.).

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[10] In accordance with 21 CFR 860.7(e).

[11] In accordance with 21 CFR 860.7(d)(1).

   a. Based on the available data, should the Epi proColon assay claims be limited to one-time screening?

      i. If no, please discuss whether a longitudinal study should be required to address long-term safety and effectiveness.

      ii. If yes, please advise if a longitudinal study should be optional.

   b. The Sponsor has proposed a warning (i.e., 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 colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.). Does this adequately address considerations (e.g., time interval and testing method) in product labeling to assure safety and effectiveness for follow-up evaluation of patients testing negative with Epi proColon?

4. Please note that the inclusion of questions related to a post-approval study should not be interpreted to mean that FDA has made a decision or is making a recommendation on the approvability of this PMA. The presence of a post-approval study plan or commitment does not in any way alter the requirements for pre-market approval and a recommendation from the Panel. The premarket data must reach the threshold for providing reasonable assurance of safety and effectiveness before the device can be found approvable and any post-approval study could be considered.

   Assuming that a longitudinal study is needed to evaluate performance with Epi proColon, please comment on the following:

   a. Is comparison to a recommended CRC screening option (e.g., annual FIT) needed to evaluate study results and to mitigate study limitations as currently proposed by the sponsor (such as controlling for incident CRC cases, lack of objective criteria for evaluating study results)?

   b. Is the proposed post-approval study adequate to address the following issues?

      i. Performance (e.g., number of test negative to positive conversions, diagnostic yield of significant findings, predictive values, adherence to screening and diagnostic follow-up);

      ii. Performance across different clinicopathologic characteristics;

      iii. Safety concerns (e.g., in the sponsor’s proposal, subjects would forgo annual FIT screening during the study duration and repeat Epi proColon testing will occur annually);

      iv. Appropriate study population (e.g., general average risk population vs. average risk population who are unwilling, unable or do not undergo screening by other recommended screening methods).

   c. Are there any additional considerations that should be taken into account for the post-approval study?

8.2 Questions for Ballot Vote

The following questions relate to the approvability of Epi proColon.

1. Is there reasonable assurance that Epi proColon is safe for use in patients who meet the criteria specified in the proposed intended use?

2. Is there reasonable assurance that Epi proColon is effective for use in the patients who meet the criteria specified in the proposed intended use?

3. In patients who meet the criteria specified in the proposed intended use, do the benefits outweigh the risks for use of Epi proColon?

FDA looks forward to a productive Panel discussion regarding these issues.

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10 APPENDICES

10.1 Cross Reactivity for Medications and Other Disease Conditions

The performance of Epi proColon was evaluated in subjects taking common medications (Table 27), subjects with chronic conditions (Table 28), and subjects with non-CRC cancers (Table 29). Some patients were reported to be on multiple medications and/or have multiple conditions and, therefore, were counted in each relevant category. There were 193 chronic disease cases and 201 cancer cases, of which 191 and 195 had valid results, respectively.

Besides the small number effects, none of the medication categories had positive detection fractions significantly different from the overall proportion of positive test results (χ^2 test p-value = 0.73). Similar results were obtained for the chronic conditions (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 pivotal and supplemental clinical studies.

There were limited numbers of subjects in each cancer category, except for breast, lung, and prostate cancers. Thus, the proportion of test positives is largely due to findings in lung and prostate cancer, which had positivity rates of 54% and 25%, respectively. The positivity rate in patients with lung cancer is substantially larger than that observed for the non-CRC groups in the pivotal clinical study (p-value < 0.001, PDF difference 32.3%, 95%CI: 21.7%, 42.9%) and supplemental clinical studies (p-values < 0.001, PDF difference 35.0%, 95%CI: 23.1%, 47.0%).

Table 27. Positive Detection Fraction Medication Category in Non-Tumor Subjects

Medication	Total	Valid	Neg	Pos	PDF (%)	95% CI
Adrenal cortical steroids	4	4	4	0	0	0, 49
Analgesics	9	9	9	0	0	0, 30
Angiotensin converting enzyme inhibitors	54	54	46	8	15	8, 27
Antidepressants	6	6	5	1	17	1, 56
Antidiabetic agents	11	11	10	1	9	0, 38
Antihistamines	2	2	1	1	50	3, 97
Antihyperlipidemic agents	26	26	22	4	15	6, 34
Antiplatelet agents	22	22	17	5	23	10, 43
β-adrenergic blocking agents	41	41	32	9	22	12, 37
Bronchodilators	8	8	5	2	25	7, 59
Calcium channel blocking agents	11	11	8	3	27	10, 57
Dermatological agents	1	1	1	0	0	0, 95
Diuretics	17	17	15	2	12	3, 34
Ophthalmic preparations	3	3	3	0	0	0, 56
Proton pump inhibitors	22	22	16	6	27	13, 48
Sex hormones	11	11	8	3	27	10, 57
Thyroid drugs	23	23	22	1	4	0, 21
Vitamins & minerals	17	16	12	4	25	10, 49
None	58	57	45	12	21	12, 33

Table 28. Positive Detection Fraction 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

Table 29. Positive Detection Fraction by Tumor Category

Cancer	Total	Valid	Neg	Pos	PDF (%)	95% CI
Colorectal (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

10.2 Reproducibility

Variability from different sources was assessed by testing 14 clinical sample pools at three sites with six operators using three reagent lots and three PCR instruments. Six pools (Pools 1-6) were generated from CRC plasma, three pools (Pools 7-9) were from healthy blood donors (self-declared), 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 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%). The Gini Index was also calculated to indicate the probability that a pair of test results for the same sample disagree (Table 30).

Table 30. Results from Reproducibility Study

Pool	Group	Total	Pos		Gini Index
			n	%	
1	CRC	12	12	100	0
2	CRC	12	12	100	0
3	CRC	12	12	100	0
4	CRC	12	12	100	0
5	CRC	12	12	100	0
6	CRC	12	12	100	0
7	NED	12	3	25	0.375
8	NED	12	3	25	0.375
9	NED	12	3	25	0.375
10	Diluted CRC	12	12	100	0
11	Diluted CRC	12	12	100	0
12	Diluted CRC	12	12	100	0
13	Diluted CRC	12	11	92	0.153
14	Diluted CRC	12	11	92	0.153

The results from the reproducibility study are below (Tables 31 and 32). The mSEPT9 results for pools 7, 8 and 9 are not shown due to the limited number of Ct values observed.

Table 31. Reproducibility Results for mSEPT9

Pool	Mean Ct	Variance Component - SD (% CV)						
		Batch	Day*	Operator	Kit	Site	Residual	Total
1	36.96	0.47 (1.28)	-	0.11 (0.30)	0 (0)	0.95 (2.58)	0.94 (2.54)	1.42 (3.85)
2	36.39	0.23 (0.64)	-	0.36 (0.99)	0.48 (1.32)	0.57 (1.56)	1.00 (2.76)	1.32 (3.63)
3	34.16	0 (0)	-	0.28 (0.82)	0.17 (0.51)	0.62 (1.82)	0.45 (1.32)	0.83 (2.44)
4	29.81	0.27 (0.92)	-	0 (0)	0 (0)	0.19 (0.65)	0.23 (0.76)	0.40 (1.36)
5	36.14	0.11 (0.30)	-	0 (0)	0.95 (2.62)	0.54 (1.50)	0.90 (2.49)	1.42 (3.92)
6	30.37	0.26 (0.86)	-	0 (0)	0 (0)	0.42 (1.38)	0.22 (0.73)	0.54 (1.79)

Table 31 continued. Reproducibility Results for mSEPT9

Pool	Mean Ct	Variance Component - SD (% CV)						
		Batch	Day*	Operator	Kit	Site	Residual	Total
10	38.85	0 (0)	0.81 (2.09)	0 (0)	0 (0)	1.48 (3.81)	1.05 (2.69)	1.99 (5.11)
11	39.52	0 (0)	0.45 (1.14)	0 (0)	0 (0)	0 (0)	1.52 (3.84)	1.58 (4.01)
12	36.89	0 (0)	0.79 (2.15)	0 (0)	0 (0)	0 (0)	1.48 (4.01)	1.68 (4.55)
13	38.65	0 (0.01)	0 (0.01)	0 (0)	0 (0)	0.95 (2.45)	2.11 (5.45)	2.31 (5.97)
14	38.59	0 (0)	0.52 (1.34)	0 (0)	0 (0)	1.01 (2.62)	1.00 (2.59)	1.51 (3.92)

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

Table 32. Reproducibility Results for ACTB

Pool	Mean Ct	Variance Component - SD (% CV)						
		Batch	Day*	Operator	Kit	Site	Residual	Total
1	27.54	0.17 (0.62)	-	0 (0)	0 (0)	0 (0)	0.11 (0.38)	0.20 (0.73)
2	26.13	0.22 (0.83)	-	0 (0)	0 (0)	0 (0)	0.07 (0.25)	0.23 (0.87)
3	27.59	0.21 (0.77)	-	0 (0)	0.08 (0.28)	0.09 (0.34)	0.10 (0.35)	0.26 (0.96)
4	27.20	0.22 (0.80)	-	0 (0)	0 (0)	0 (0)	0.13 (0.47)	0.25 (0.93)
5	27.32	0.05 (0.17)	-	0.06 (0.21)	0.21 (0.77)	0 (0)	0.17 (0.62)	0.28 (1.02)
6	27.47	0.23 (0.83)	-	0 (0)	0.08 (0.28)	0 (0)	0.14 (0.51)	0.28 (1.02)
7	27.14	0.17 (0.61)	-	0 (0)	0.08 (0.28)	0 (0)	0.19 (0.67)	0.27 (0.95)
8	27.79	0.28 (0.99)	-	0 (0)	0 (0)	0.20 (0.71)	0.09 (0.34)	0.35 (1.27)
9	28.13	0.18 (0.62)	-	0 (0)	0 (0)	0 (0)	0.10 (0.37)	0.20 (0.73)
10	26.76	0.10 (0.39)	0 (0)	0.16 (0.59)	0 (0)	0 (0)	0.28 (1.04)	0.31 (1.26)
11	23.22	0 (0)	0 (0)	0.23 (0.99)	0.11 (0.46)	0 (0)	0.27 (1.16)	0.37 (1.59)
12	26.99	0 (0)	0.16 (0.60)	0.17 (0.64)	0 (0)	0 (0)	0.12 (0.46)	0.27 (0.99)
13	26.61	0.04 (0.14)	0 (0)	0.20 (0.74)	0 (0)	0 (0)	0.15 (0.57)	0.25 (0.94)
14	25.07	0 (0)	0.26 (1.04)	0 (0)	0 (0)	0 (0)	0.10 (0.41)	0.28 (1.11)

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

10.3 Risk-Benefit Analysis

10.3.1 Additional True and False Positives

To demonstrate the potential benefits and risks, the diagnostic yield from both tests in a population of 100,000 subjects was projected under the assumption that CRC prevalence is 0.7% based on the PRESEPT study. Under this circumstance, 700 subjects (among 100,000) are expected to have CRC. When the prevalence is applied to the sensitivity for CRC and specificity for non-CRC observed in the study (72.2% and 68.0%, respectively), the numbers of true positives and false negatives can be determined for each test (Table 33).

Table 33. Projected True Positives Among 100,000 Subjects

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

Comparing the results for both the CRC and non-CRC projected populations, Epi proColon can be expected to detect 29 more CRC subjects than the FIT test used in this study, as well as 16,464 additional non-CRC subjects. This means that for each additional true positive result, there will be 571 additional false positives.

10.3.2 Additional Adverse Events

The risk of an adverse event resulting from a follow-up colonoscopy from a false positive test result can also be projected. Assuming that the risk of an adverse event resulting from a follow-up colonoscopy is 0.68% (Rutter CM, 2012)[12], 18 adverse events (2573*0.0068) can be expected from a follow-up colonoscopy referred after a false positive result by the FIT test used in this study. For follow-up colonoscopies after a false positive Epi proColon test, 130 adverse events (19037*0.0068) are expected. In comparison to the FIT test, there are expected to be 112 additional adverse events from a colonoscopy after a false positive Epi proColon test.

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[12] This retrospective cohort study included individuals from 40 to 85 years old.