Epi proColon®

Epi proColon Plasma Quick Kit (M5-02-001)

Epi proColon Sensitive PCR Kit (M5-02-002)

Epi proColon Control Kit (M5-02-003)

Rx Only

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
For *in vitro* diagnostic use only.
Read and follow all instructions for use prior to using this test.

REF M5-02-001, M5-02-002, M5-02-003

Contents

1. Intended Use, Contraindications, Warnings, Precautions and Limitations

Intended Use

The Epi proColon test is a qualitative in vitro diagnostic test for the detection of methylated Septin 9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the *SEPT*9_v2 transcript has been associated with the occurrence of colorectal cancer (CRC). The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin 9 DNA target.

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

Contraindications

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

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

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

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

Warnings, Limitations and Precautions

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

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

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

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

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

- The rate of false positive Epi proColon results increases with age. Test results should be interpreted with caution in elderly patients. See Performance Characteristics in Section 13.

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

- Positive test results have been observed in healthy subjects and in patients diagnosed with chronic gastritis, lung cancer, and in pregnant women. [6,7]

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

2. Summary and Explanation

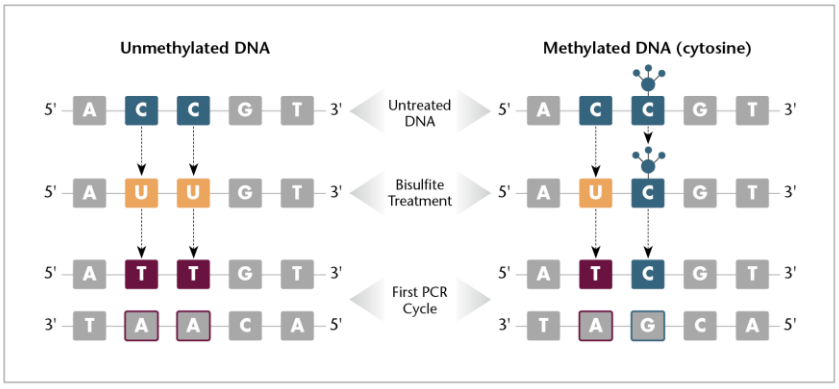
The Epi proColon test is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of methylated Septin 9 DNA isolated from 3.5 mL of patient plasma. There is an increased methylation of cytosine residues in the *v*2 region of the Septin 9 gene in colorectal cancer (CRC) tissue. This aberrant methylation can be detected by specific amplification of Septin 9 DNA present in the plasma sample. Detection of CRC DNA in plasma using the methylated Septin 9 DNA biomarker has been demonstrated in multiple case control studies of CRC patients and colonoscopy-verified negative controls[1-4], as well as in two multicenter clinical evaluations. The Epi proColon blood test offers patients who have a history of non-adherence to other recommended methods and who decline guideline recommended tests, an alternative option to participate in a CRC screening program.

3. Principles of the Procedure

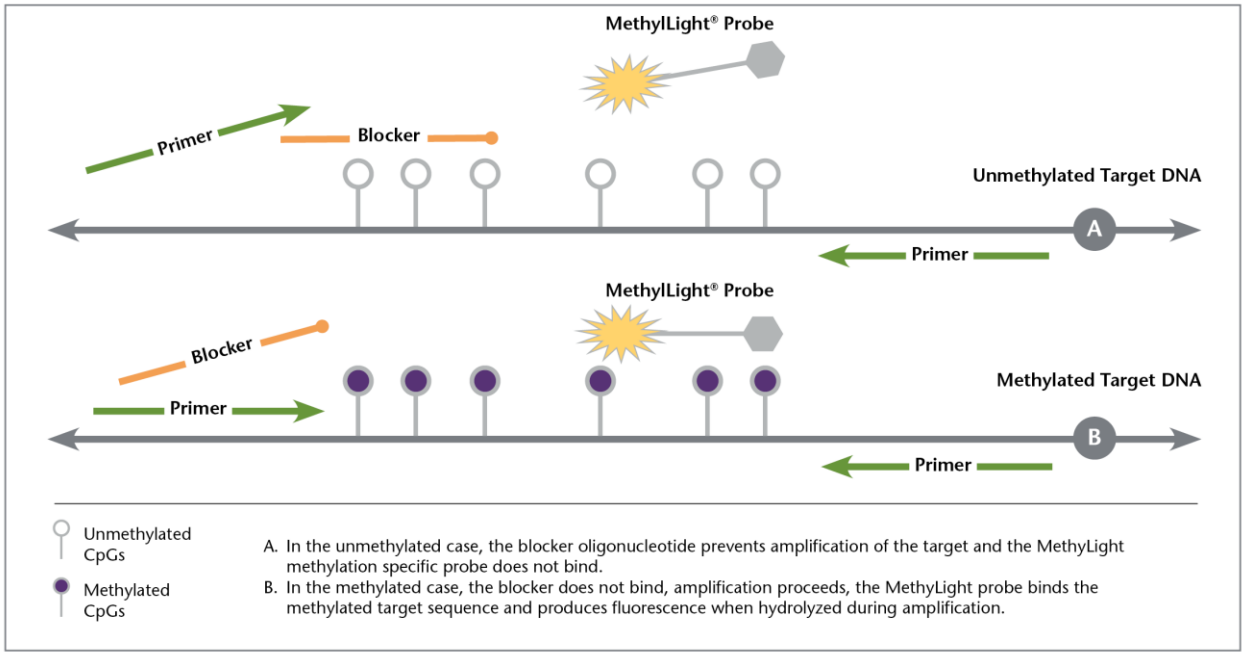
The Epi proColon test involves two primary procedural phases:

First, DNA is extracted from plasma and treated with bisulfite using the Epi proColon Plasma Quick Kit (M5-02-001). DNA is extracted from patient plasma by binding to magnetic beads. Impurities are removed from the magnetic beads by a wash step. The purified DNA is then released from the beads by suspension in an elution buffer, and treated with bisulfite reagents to produce bisulfite converted DNA (bisDNA). The bisDNA is then re-purified using magnetic beads. Bisulfite conversion is the method of choice for analyzing DNA methylation. It is based on the nucleophilic addition of a bisulfite ion to a cytosine nucleotide and a subsequent deamination reaction to yield uracil sulfonate; 5-methylcytosine (methylated cytosine) is protected from deamination by the methyl group. Thus, the sequence of bisDNA differs from the input DNA by the substitution of uracil nucleotides for unmethylated cytosines, while retaining methyl cytosine bases.

Second, bisDNA is assayed by a duplex Real-Time PCR (M5-02-002) with simultaneous detection of the target methylated Septin 9 DNA and the internal control ACTB (ß-actin) DNA. The control is designed to establish that sufficient input DNA is included in the reaction. Epi proColon Positive and Epi proColon Negative Controls are provided with the Epi proColon Control Kit (M5-02-003) and are required for each test run. The Epi proColon test detects a



bisDNA sequence containing methylated CpG sites within the *v*2 region of the *SEPT9* gene and a bisDNA sequence from the ACTB gene with no methylated CpG sites. The Epi proColon test discriminates methylated cytosines from cytosines using a combination of a blocker oligonucleotide and a methylation specific probe. The reaction comprises primers that are placed in regions lacking CpG dinucleotides, a blocker specific for bisulfite converted unmethylated sequences within the region is added to preferentially amplify methylated sequences, and a methylated Septin 9 specific fluorescent detection probe to exclusively identify methylated sequences amplified during the duplex PCR reaction[5].



4. Precautions and Safety

Laboratory Precautions Related to Real-Time PCR

- The Epi proColon test is for *in vitro* diagnostic use only
- This procedure is for professional laboratory use only and assumes familiarity with DNA extraction methods and real time 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 stageCompliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples during and after the DNA extraction, bisulfite conversion, and purification procedure
- Use only single-use pipettes and filter tips to prevent cross-contamination of the patient sample
- Use of reference pipettes for pipetting extracted and bisulfite treated DNA is strongly recommended
- Good technique is important to prevent the introduction of nucleases into samples during the extraction procedure

- Do not freeze extracted DNA
- Epi proColon Bisulfite Solution is sensitive to oxygen contact; use only unopened tubes of Epi proColon Bisulfite Solution; do not store but discard any left-over solution
- When removing liquid from microtubes in multiple steps in the procedure, take care not to remove magnetic beads
- In step 9.12, the "dry" spin step is very important. If the remaining droplets are not removed from the beads before drying and elution of the DNA, the remaining Wash B Buffer can be the cause of an INVALID PCR result.
- Strict separation of pre-PCR activities (e.g., plasma DNA extraction and purification, PCR setup) and post-PCR activities (e.g., Real-Time PCR) is highly recommended to prevent contamination by amplicons generated from previous PCR testing.
- To prevent the release of any PCR product, used PCR plates should never be opened. Place the PCR plate in a resealable plastic bag immediately after removal from the PCR instrument, close and dispose the bag in a dedicated PCR waste container.

Additional Precautions

- Do not mix kit components between kit lots
- Do not use kits or kit components beyond their stated expiration date
- BD Vacutainer® K2EDTA blood collection tubes should be allowed to complete the evacuated fill to ensure the required 3.5 mL of plasma is obtained post-centrifugation
- Do not freeze BD Vacutainer K2EDTA blood collection tubes or whole blood collected in these tubes
- The Epi proColon test kits do not contain infectious substances or agents that may cause disease in humans or animals
- All patient blood and plasma specimens should be handled as though they are capable of transmitting disease. Observe universal precautions and safe laboratory procedures as specified in the OSHA Standard on Bloodborne Pathogens, CLSI Document M29-A3, and any other appropriate biosafety practices as required by your laboratory.

Safety Information

When working with chemicals, always wear a laboratory coat and disposable gloves. Clean contaminated surfaces with water. For more information, please refer to the respective Safety Data Sheets (SDS) available at the E•Library found at epiprocolon.com.

Epi proColon Lysis Binding Buffer and Epi proColon Wash A Concentrate: Contain TRITON X-100 and Guanidinium thiocyanate.

Hazard statements: Harmful if swallowed. Harmful in contact with skin. Harmful if inhaled. Causes skin irritation. Causes serious eye damage.

Precautionary statements:

Prevention: Avoid breathing mist/vapors/spray. Wash thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Wear protective gloves/protective clothing/eye protection/face protection.

DANGER

Response: If swallowed: Call a poison center/doctor if you feel unwell. Rinse mouth. If on skin: Wash with plenty of water. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a

poison center/doctor. If inhaled: Remove person to fresh air and keep comfortable for breathing. Call a poison center/doctor if you feel unwell.

Storage: Store away from incompatible materials.

Disposal: Dispose of contents/container in accordance with local/ regional/ national/ international regulations.

Epi proColon Bisulfite Solution: Contains aqueous solution of ammonium bisulfite (Ammonium hydrogen sulfite).

Hazard statements: Causes serious eye irritation.

Precautionary statements:

Prevention: Wash thoroughly after handling. Wear eye protection/face protection.

Response: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

Storage: None.

Disposal: None.

WARNING

Epi proColon Protection Buffer: Contains 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Tetrahydrofurfurylalcohol.

Hazard statements: Combustible liquid. Causes skin irritation. Causes serious eye irritation. May damage fertility or the unborn child.

Precautionary statements:

Prevention: Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Keep away from heat/sparks/open flames/hot surfaces. - No smoking. Wash thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection.

Response In case of fire: Use foam, carbon dioxide, dry powder or water fog for extinction. If on skin: Wash with plenty of water. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

Storage Store in a well-ventilated place. Keep cool. Store locked up.

Disposal Dispose of contents/container in accordance with local/ regional/ national/ international regulations.

DANGER

Epi proColon Wash B Concentrate, Epi proColon Elution Buffer, Epi proColon Magnetic Beads, Epi proColon PCR Mix, Epi proColon Polymerase, Epi proColon Positive Control, and Epi proColon Negative Control are not harmful.

5. Materials Provided

The Epi proColon test is comprised of the Epi proColon Plasma Quick Kit (M5-02-001), the Epi proColon Sensitive PCR Kit (M5-02-002) and the Epi proColon Control Kit (M5-02-003).

Contents

Table 1: Contents of the Epi proColon Plasma Quick Kit (M5-02-001)

Reagent	Containers	Volume	Storage Temperature
Epi proColon Lysis Binding Buffer	1 bottle	125 mL	15°C to 30°C
Epi proColon Wash A Concentrate	1 bottle	60 mL	15°C to 30°C
Epi proColon Magnetic Beads	1 bottle	4 mL	15°C to 30°C
Epi proColon Wash B Concentrate	1 bottle	7 mL	15°C to 30°C
Epi proColon Elution Buffer	1 tube	6 mL	15°C to 30°C
Epi proColon Bisulfite Solution	4 tubes	1.9 mL each	15°C to 30°C
Epi proColon Protection Buffer	1 tube	1 mL	15°C to 30°C

Note: The color of the Epi proColon Protection Buffer can vary from a clear to a brownish color.

Table 2: Contents of the Epi proColon Sensitive PCR Kit (M5-02-002)

Reagent	Containers	Volume	Storage Temperature
Epi proColon PCR Mix	2 tubes	810 µl each	-25°C to -15°C
Epi proColon Polymerase	1 tube	85 µl	-25°C to -15°C

Table 3: Contents of the Epi proColon Control Kit (M5-02-003)

Reagent	Containers	Volume	Storage Temperature
Epi proColon Negative Control	6 tubes	3.65 mL each	-25°C to -15°C
Epi proColon Positive Control	6 tubes	3.65 mL each	-25°C to -15°C

6. Storage and Stability

Reagents provided with the Epi proColon Plasma Quick Kit (M5-02-001), Epi proColon Sensitive PCR Kit (M5-02-002) and Epi proColon Control Kit (M5-02-003) are stable until the expiration date when stored and handled as directed. Do not use material past expiration date. Do not mix components from different kit lots.

Epi proColon Plasma Quick Kit (M5-02-001)

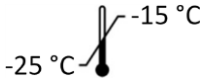
Store all reagents of the Epi proColon Plasma Quick Kit at 15 to 30 °C.

Epi proColon Bisulfite Solution is sensitive to oxygen contact. Use only unopened tubes of Epi proColon Bisulfite Solution. Discard used tubes!

Store reconstituted Epi proColon Wash A Buffer and reconstituted Epi proColon Wash B Buffer at 15 to 30 °C for up to 6 weeks.

After first use store all reagents at 15 to 30 °C for up to 6 weeks.

Epi proColon Sensitive PCR Kit (M5-02-002)



Store Epi proColon PCR Mix and Epi proColon Polymerase at -25 to -15 °C.

Each Epi proColon PCR Mix tube may be thawed and refrozen one time. After first use store all reagents at -25 to -15 °C for up to 6 weeks.

Epi proColon Control Kit (M5-02-003)

Store Epi proColon Control Kit at -25 to -15 °C.

7. Materials Required But Not Provided

The following general laboratory equipment is required to perform the Epi proColon test. All laboratory equipment should be installed, calibrated, operated, and maintained according to the manufacturer's recommendations.

The following tables for required and special equipment and consumables are provided along with suggested vendors and catalog and part numbers.

General Laboratory Equipment

Required Equipment	Suggested Provider
Tube racks for 15 mL tubes and 2 mL tubes	Fisher Scientific, Cat. No. 03-448-11, or equivalent
Rotator with adjustable angle	GLAS-COL 099A RD4512, VWR International, Cat. No. 62404-006, or equivalent
Vortex Mixer	Thermo Scientific MaxiMix II vortex mixer, Cat. No. M37615Q, or equivalent
Thermoshaker for 2 mL tubes	Thermomixer® C with dry heating block 2 mL Eppendorf, Cat. No. 5382000023 and 5362000035or equivalent
Magnetic Separator	DynaMag™-15 magnet, Life Technologies, Cat. No. 12301D
Magnetic Separator	DynaMag™-2 magnet, Life Technologies, Cat. No. 12321D
Reference Pipettes with adjustable volumes of the following ranges 10-100 µL, 100-1000 µL	Eppendorf Research® Plus, adjustable-volume pipette, Eppendorf, Cat. No. 3120000046, 3120000062, or equivalent
Repeat Pipettor capable of repetitively dispensing volumes in an adjustable range	Eppendorf Repeater® M4, Eppendorf, Cat. No. 4982000322, or HandyStep electronic, Brandtech®, Cat. No. 705012, or equivalent
Bench Top Centrifuge with rotor for 1.5/2.0 mL tubes	Centrifuge 5430 with Rotor FA-45-30-11, Eppendorf, Cat. No. 022620509 or equivalent
Multichannel Pipette	Eppendorf 8-Channel, 10-100 µL Cat. No. 3122000035, or equivalent
Centrifuge for PCR plates	Centrifuge 5430 (Bench Top above) Eppendorf Cat. No. 022620509 with rotor 022654403, or Centrifuge 4-16S, Qiagen Inc., Cat. No. 81510 and 81031, or equivalent
100 mL graduated cylinder (PP)	Brandtech®, Cat. No. V649941, or equivalent

General Laboratory Consumables and Reagents

Consumables and Reagents	Suggested Consumables and Reagents
Ethanol absolute, 200 proof, for molecular biology, ≥99.5 %	Sigma-Aldrich Co., Cat. No. E7023, or equivalent
15 mL polypropylene (PP) centrifuge tubes with conical bottom, sterile	Sarstedt, Cat. No. 62.554.502, or equivalent
2.0 mL microtubes with round bottom and with attached PP cap with lid seal mechanism	Sarstedt SafeSeal, Cat. No. 72.695.400, or equivalent
Pipette tips with aerosol barrier	Eppendorf ep Dualfilter T.I.P.S®: - 2-100 µL Cat. No. 022491237, or equivalent - 50-1000 µL Cat. No. 022491253, or equivalent
Repeat Pipettor Tips for volumes of 0.5 mL, 1 mL, 10 mL, 25 mL	Eppendorf Combitips Advanced® : - 0.5 mL Cat. No. 0030089421, or equivalent - 1 mL Cat. No. 0030089430, or equivalent - 10 mL Cat. No. 0030089464, or equivalent - 25 mL Cat. No. 0030089472, or equivalent
Disposable Transfer Pipettes, non-sterile bulk packaged, length about 15 cm (6 inches), stem diameter 5 mm, capacity about 5 mL	Samco™, graduated 1 mL large bulb non-sterile pipettes, Thermo Scientific Cat. No. 222, or equivalent
Disposable Transfer Pipettes, non-sterile bulk packaged, length about 23 cm (9 inches), stem diameter 5 mm, capacity about 5 mL	Samco™ extra-long transfer pipettes, Thermo Scientific, Cat. No. 262, or equivalent
96 well plates for DNA storage	PCR plate 96, skirted, Eppendorf, Cat. No. 951020401, or equivalent
Adhesive Film or Foil for DNA storage plate	VWR® PCR sealing film Cat. No. 82018-844, or equivalent
Applicator to form a tight seal between a microplate and an adhesive film	MicroAmp® adhesive film applicator, Life Technologies, Cat. No. 4333183, or equivalent

Consumables and Reagents	Suggested Consumables and Reagents
Cryo vials, 4 mL	VWR Cat. No. 89094-812, or equivalent
Resealable bags, 10 x 15 cm, for disposing of used PCR plates	VWR Cat. No. 89005-280, or equivalent

Required Special Equipment and Consumables

The following special equipment and consumables are needed to perform the Epi proColon test and cannot be replaced by other equipment.

Special Equipment/Consumables	Catalog and Part Numbers
Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with Sequence Detection Software v1.4 21 CFR Part 11 Module and Windows XP operating system with Service Pack 2 or 3	Life Technologies (Thermo Fisher Scientific, Inc.) part no. 4406984 or 4406985
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	Applied Biosystems (Life Technologies Co.), 20 plates, Cat. No. 4346906, 200 plates, Cat. No. 4366932
MicroAmp® 96- & 384-Well Optical Adhesive Film	Applied Biosystems (Life Technologies, Thermo Fisher Scientific, Inc.) 25 sheets, Cat. No. 4360954 or 100 sheets, Cat. No. 4311971
Vacutainer® K2EDTA 10 mL Blood Collection Tubes	Becton Dickinson, Cat. No. 366643

Installation Requirements

The installation, calibration, performance verification and maintenance of the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument must be performed according to the manufacturer's instructions.

Note: Monthly background calibration as described in the manufacturer's maintenance procedure must be performed by the laboratory. Furthermore the semi-annual maintenance tasks Operation Qualification and Performance Qualification (OQ/PQ) including calibration of the pure dyes FAM™,JOE™, TAMRA™ and the annual maintenance must be performed by an Applied Biosystems' Field Service Engineer.

8. Specimen Collection and Handling

Blood Collection

Blood should be collected according to your laboratory's procedure for venipuncture using **ONLY Vacutainer® K2EDTA 10 mL blood collection tubes (lavender top, Becton Dickinson)**. **Blood collection tubes should be allowed to complete the evacuated fill. Whole blood may be stored refrigerated or at room temperature (**2°C to 30°C). **Plasma preparation should be performed within 4 hours after blood is collected**. **Ensure a minimum of 3.5 mL of plasma is obtained following centrifugation.**

The plasma sample may be stored refrigerated, at 2°C to 8°C for up to 72 hours, or frozen, at -15°C to -25°C for up to 14 days. Transport and shipment of refrigerated or frozen plasma should follow the same temperature and time requirements as stated above.

Note: Do not freeze whole blood samples.

Plasma Specimen Preparation

- Label all tubes with appropriate patient identification information
- Prior to use, disable the brake function in the centrifuge to prevent disruption of the cell layer
- Centrifuge the Vacutainer K2EDTA blood collection tube for 12 min at 1350 ± 150 rcf.
  [For conversion of RPM (revolutions per minute) to rcf (relative centrifugal force), refer to the centrifuge manufacture's user manual]
- Remove blood collection tube from the centrifuge (If the plasma sample is hemolyzed, discard and acquire a new blood specimen from the patient)
- Using a fresh 6 inch disposable transfer pipette, transfer plasma from the blood collection tube to a 15 mL polypropylene centrifuge tube with conical bottom
- Centrifuge plasma in the 15 mL centrifuge tube for 12 min at 1350 ± 150 rcf
- Using a fresh, extra-long 9 inch disposable transfer pipette or serological pipette, transfer a minimum of 3.5 mL of plasma into a labelled cryovial or centrifuge tube
- Start DNA extraction with the Epi proColon Plasma Quick Kit
- Alternatively, in a labelled cryovial, store plasma at 2°C to 8°C for up to 72 hours. For storage for up to 14 days, store the plasma at -15°C to -25°C.

Note: Take care not to disturb or transfer the buffy coat (white blood cells) layered above the red blood cells in the blood collection tube after the first centrifugation or sedimented at the bottom of the conical centrifuge tube after the second centrifugation.

9. Test Procedure

A repeat pipettor is recommended for repetitive dispensing of the following reagents:

- Epi proColon Lysis Binding Buffer
- Ethanol in the lysis binding step 9.3

Reference pipettes are recommended for the following:

- Epi proColon Magnetic Bead suspension
- Ethanol in DNA binding step 9.4
- Epi proColon Wash A Buffer
- Epi proColon Wash B Buffer
- Epi proColon Elution Buffer
- Epi proColon Bisulfite Solution
- Epi proColon Protection Buffer and the PCR Master Mix

Use of reference pipettes for pipetting extracted and bisulfite treated DNA is strongly recommended.

9.1. Preparation of Working Solutions

Preparation of Epi proColon Wash A Buffer

- Add 60.0 mL of Absolute Ethanol (for molecular biology, ≥99.5 %) to the Epi proColon Wash A Concentrate using a sterile graduated cylinder or a serological pipette

- Close lid, mix thoroughly by inverting the bottle five times, avoiding formation of foam; Label the bottle with date of dilution, and mark the “Ethanol added” checkbox
- Store reconstituted Epi proColon Wash A Buffer at 15°C to 30°C for up to 6 weeks

Preparation of Epi proColon Wash B Buffer

- Add 40.0 mL of Absolute Ethanol (for molecular biology, ≥99.5 %) to the Epi proColon Wash B Concentrate using a sterile graduated cylinder or a serological pipette
- Close lid, mix thoroughly by inverting the bottle five times. Label the bottle with date of dilution, and mark the “Ethanol added” checkbox
- Store reconstituted Epi proColon Wash B Buffer at 15°C to 30°C for up to 6 weeks

9.2. DNA Extraction and Bisulfite Conversion from Patient Plasma

The Epi proColon test kit contains sufficient reagents to process up to 32 samples including quality controls. One Epi proColon Positive Control and one Epi proColon Negative Control must be included in each independent test run. There are four single-use tubes of Epi proColon Bisulfite Solution that will enable a maximum of four independent test runs (e.g., 4 runs of 8 samples each).

Note: Brief centrifugation of microtubes (stated as ‘Briefly spin down the tubes‘) is required in several steps of this instruction to remove drops from the lid and/or to collect remaining liquid. It is recommended to centrifuge for 10 to 20 sec at 1,000 ± 150 rcf using a Bench-Top centrifuge. Avoid stronger centrifugation to prevent the compacting of magnetic bead pellets in these steps. For conversion of RPM (revolutions per minute) to rcf (relative centrifugal force), refer to the manufacturer’s centrifuge user manual.

Note: Vortexing of tubes and vials is required in several steps of this instruction to ensure homogeneous mixing of liquid. It is recommended to vortex samples for 5 to 10 sec at medium speed.

Thawing of Plasma and Epi proColon Positive and Negative Control

- Thaw one Epi proColon Positive Control and one Epi proColon Negative Control for 30 min at 15°C to 30°C
- If frozen plasma samples are used, thaw samples for about 30 min at 15°C to 30°C
- Start lysis within 60 min after thawing

9.3. Lysis

Note: Prior to use, briefly shake the Epi proColon Lysis Binding Buffer and visually check for precipitates. If precipitates are present heat the Epi proColon Lysis Binding Buffer in a water bath at 37°C for 60 min and shake gently until the precipitate is completely dissolved. Equilibrate Epi proColon Lysis Binding Buffer to room temperature before use.

- Add the following to a labelled 15 mL conical centrifuge tube:
  - 3.5 mL plasma sample, Epi proColon Positive Control, or Epi proColon Negative Control
  - 3.5 mL Epi proColon Lysis Binding Buffer
- Cap the tube and vortex for 5 to 10 sec
- Incubate tubes on the bench top at 15°C to 30°C for 10 ± 1 min

9.4. DNA Binding

Note: A homogeneous suspension of the Epi proColon Magnetic Beads is essential for proper test performance. Deviations from the specified amount of beads may lead to false results. To ensure the

correct magnetic bead concentration, the bottle should be mixed thoroughly just before pipetting. There should be no visible sediment at the bottom of the bottle. Mix to ensure a homogeneous suspension between the pipetting steps.

- Add to the 15 mL centrifuge tube in the following order:
  - 90 µL Epi proColon Magnetic Beads (freshly suspended)
  - 2.5 mL of Absolute Ethanol (for molecular biology, ≥99.5 %)
- Cap the tube and mix by inverting the tube 5 to 6 times
- Place 15 mL tubes into a rotator
- Rotate at room temperature for 45 ± 5 min at medium speed (approximately 10 to 20 rpm); adjust rotator angle to 35 to 45 degrees

9.5. DNA Washing

Note: Before starting the wash procedure set the thermoshaker to 80 ± 2°C for later use in the elution and bisulfite conversion steps.

- Place the 15 mL tubes into the DynaMag™-15 magnetic racks for 5 to 10 min
- Pour off the supernatant carefully, taking care not to remove magnetic beads
  - Add 1.5 mL Epi proColon Wash A Buffer
- Resuspend magnetic beads completely by vortexing for 5 to 10 sec
- Using an extra-long (9 inch) disposable transfer pipette, transfer magnetic beads suspension into a labelled 2.0 mL microtube
- Place disposable transfer pipette back into 15 mL tube to collect remaining magnetic beads and transfer them into the 2.0 mL microtube
- Place the microtubes into the DynaMag-2 magnetic racks for 2 to 6 min
- Using a 6 inch disposable transfer pipette, remove as much buffer as possible while microtubes are still in the DynaMag-2 magnetic rack, taking care not to remove magnetic beads
- Briefly spin down the microtubes
- Place the 2.0 mL microtubes into the DynaMag-2 magnetic racks for 2 to 6 min
- Using a 10-100 µL reference pipette, remove as much residual buffer as possible while microtubes are still in magnetic rack

9.6. Elution

- Transfer the microtubes into a non-magnetic rack
- Vortex Epi proColon Elution Buffer for 5 to 10 sec
- Add 100 µL Epi proColon Elution Buffer to each microtube
- Close the microtubes
- Resuspend the magnetic beads by vortexing for 5 to 10 sec
- Place microtubes into a thermoshaker set to 1,000 ± 100 rpm and incubate at 80 ± 2°C for 10 ± 1 min
- Briefly spin down the microtubes
- Place microtubes into the DynaMag-2 magnetic racks for 2 to 6 min.

- Transfer the complete eluate, while microtubes are still in the magnetic rack, (~100 µL DNA solution) into fresh 2.0 mL microtubes
- Discard the 2 mL microtubes containing the magnetic beads

9.7. Storage of Extracted DNA

Note: If extracted DNA is not used immediately, store material at 2°C to 8°C for up to 24 hours. DO NOT FREEZE THE EXTRACTED DNA.

9.8. Bisulfite Conversion

Note: Epi proColon BISULFITE SOLUTION IS SENSITIVE TO OXYGEN CONTACT. Use only unopened tubes of Epi proColon Bisulfite Solution; do not store but discard any left-over solution.

Note: The color of the Epi proColon Protection Buffer can vary from a clear to a brownish color.

- Add the following reagents to the 2.0 mL microtubes containing the eluate (~100 µL DNA solution):
  - 150 µL Epi proColon Bisulfite Solution
  - 25 µL Epi proColon Protection Buffer
- Cap the microtubes and mix the bisulfite reaction by vortexing for 5 to 10 sec
- Briefly spin down the microtubes
- Place microtubes into thermoshaker and incubate for 45 ± 5 min at 80 ± 2°C without shaking
- Remove microtubes from the thermoshaker immediately after 45 ± 5 min
- Reset thermoshaker temperature to 23 ± 2°C, or set up a second thermoshaker to 23 ± 2°C for later use

9.9. Binding Step

Note: A homogeneous suspension of beads in the Epi proColon Magnetic Beads suspension is essential for proper performance. Deviations from the specified amount of beads may lead to false results. To ensure correct magnetic bead concentration, the bottle should be mixed thoroughly just before pipetting. There should be no visible sediment at the bottom of the bottle. Mix to ensure a homogeneous suspension between the pipetting steps.

- Briefly spin down the 2.0 mL microtubes containing the bisulfite reaction
- Add the following components to the microtube:
  - 1000 µL Epi proColon Wash A Buffer
  - 20 µL Epi proColon Magnetic Beads (freshly suspended)
- Mix by vortexing for 5 to 10 sec
- Wait until thermoshaker reaches 23 ± 2°C
- Place the microtubes in the thermoshaker at 1000 ± 100 rpm and incubate for 45 ± 5 min at 23 ± 2°C
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min.
- Using a fresh 6 inch disposable transfer pipette, remove as much liquid as possible while tubes are still in the magnetic rack, taking care not to remove magnetic beads

9.10. First Wash

- Remove the sample rack from the magnet for washing and vortexing
  - Add 800 µL Epi proColon Wash A Buffer
- Resuspend by vortexing for 5 to 10 sec
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min.
- Using a fresh 6 inch disposable transfer pipette, remove as much liquid as possible while tubes are still in the magnetic rack, taking care not to remove magnetic beads

9.11. Second Wash

- Remove the sample rack from the magnet for washing and vortexing
  - Add 800 µl Epi proColon Wash B Buffer
- Resuspend by vortexing for 5 to 10 sec
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min
- Using a fresh 6 inch disposable transfer pipette, remove as much liquid as possible while tubes are still in the magnetic rack, taking care not to remove magnetic beads

9.12. Third Wash

- Remove the sample rack from the DynaMag™-2 magnet for washing and vortexing
  - Add 400 µl Epi proColon Wash B Buffer
- Resuspend by vortexing for 5 to 10 sec
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min
- Using a fresh 6 inch disposable transfer pipette, remove as much liquid as possible while tubes are still in the magnetic rack, taking care not to remove magnetic beads
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min
- Using a 10-100 µL reference pipette, remove as much remaining liquid as possible while tubes are still in the magnetic rack, taking care not to remove magnetic beads

Note: This “dry” spin step is very important. If the remaining droplets are not removed from the Beads, remaining Epi proColon Wash B Buffer can be the cause of an INVALID PCR result.

9.13. Drying

Note: Do not increase drying time or temperature as over-drying might reduce bisDNA recovery

- Open microtube lid
- Place open microtubes into thermoshaker
- Allow the pellet to dry for 10 ± 1 min at 23 ± 2°C without shaking

9.14. Elution

- Transfer microtubes into a non-magnetic rack
  - Add 60 µL Epi proColon Elution Buffer

- Close the microtubes
- Resuspend the magnetic beads by vortexing for 5 to 10 sec
- Incubate for 10 ± 1 min at 23 ± 2°C in a thermoshaker at 1000 ± 100 rpm
- Briefly spin down the microtubes
- Place microtubes on the DynaMag™-2 magnetic racks for 2 to 6 min
- Using a 10-100 µL reference pipette, transfer the complete eluate (~ 60 µL DNA solution) into a 96-well plate and seal the plate with adhesive film using an adhesive film applicator
- Set up the bisDNA storage plate according the recommended plate layout in Table 4

Table 4: Recommended Layout for a bisDNA Storage Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC*	S7										
B	NC*	S8										
C	S1	S9										
D	S2	S10										
E	S3	S11										
F	S4	S12										
G	S5	S13										
H	S6	S14										

* PC: Epi proColon Positive Control, NC: Epi proColon Negative Control

9.15. Storage of bisDNA

If purified bisDNA is NOT USED IMMEDIATELY, store material at 2°C to 8°C for up to 24 hours or frozen at -25°C to -15°C for up to 3 days.

9.16. PCR Setup

Note: Each bisDNA sample (patient sample, Epi proColon Positive Control, or Epi proColon Negative Control) must be tested in triplicate.

Note: Prior to use, spin Epi proColon Polymerase for 10 - 20 sec at 1,000 ± 150 rcf using a Bench-Top centrifuge to remove drops from the lid.

Preparation of PCR Master Mix

- Thaw 1 or 2 Epi proColon PCR Mix tubes depending on the desired number of patient and control sample determinations, Table 5
- Vortex the Epi proColon PCR Mix tube(s) for 5 to 10 sec, briefly spin down the tube(s)
- Transfer the corresponding volumes of Epi proColon PCR Mix and Epi proColon Polymerase as indicated in Table 5 into a 2.0 mL microtube
- Mix the PCR Master Mix by vortexing for 5 to 10 sec

- Briefly spin the PCR Master Mix to remove drops from the lid

Note: Use PCR Master Mix, immediately. Do not store. Refreeze unused Epi proColon PCR Mix and Epi proColon Polymerase, directly after use.

Table 5: Preparation of PCR Master Mix

Component	Volume for 8 Determinations (24 PCRs)	Volume for 16 Determinations (48 PCRs)	Volume for 24 Determinations (72 PCRs)	Volume for 32 Determinations (96 PCRs)
Epi proColon PCR Mix	383.8 µL	767.7 µL	1151.5 µL	1535.4 µL
Epi proColon Polymerase	19.4 µL	38.7 µL	58.1 µL	77.4 µL

Note: For a single PCR, 14.3 µL Epi proColon PCR Mix and 0.7 µL Epi proColon Polymerase are required.

PCR Plate Preparation

- Set up the PCR plate. The layout as shown in Table 6 is recommended
- Transfer 15 µl PCR Master Mix into the selected wells of the MicroAmp® Fast Optical 96-Well Reaction Plate
- Briefly centrifuge the bisDNA storage plate created in section 9.14 for 1 min at 1000 ± 100 rcf using the plate centrifuge
- Add 15 µl of each bisDNA solution to 3 independent wells of the PCR plate as shown in Table 6

Table 6: Recommended PCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC*	PC*	PC*	S7	S7	S7						
B	NC*	NC*	NC*	S8	S8	S8						
C	S1	S1	S1	S9	S9	S9						
D	S2	S2	S2	S10	S10	S10						
E	S3	S3	S3	S11	S11	S11						
F	S4	S4	S4	S12	S12	S12						
G	S5	S5	S5	S13	S13	S13						
H	S6	S6	S6	S14	S14	S14						

*PC: Epi proColon Positive Control, NC:Epi proColon Negative Control

- Seal the plate with MicroAmp® Optical Adhesive Film
- Briefly spin down the plate with a plate centrifuge for 1 min at 1000 ± 100 rcf

Note: The filled PCR plate can be stored in a refrigerator at 2°C to 8°C for up to 4 h.

Plate Loading

Note: The PCR Master Mix does not contain ROX or any other reference dye. Accordingly, the passive reference setting must be set to “none”.

- Start the Software version SDS v1.4 21 CFR Part 11
- Create a new plate document
- Click “Create New Document”
- Define the following plate document:
  - Assay: Standard Curve (Absolute Quantification)
  - Container: 96-Well Clear
  - Template: Blank Document
  - Run Mode: Standard 7500
- Click “Next”
- Click “New Detector…”
- Create a new detector using following properties:
  - Name: “Septin9”
  - Description: “Epi proColon”
  - Reporter dye: “FAM”
  - Quencher dye: (none)
  - Color: “Red”
- Click “Create Another” and define following properties:
  - Name: “ACTB”
  - Description: “Epi proColon”
  - Reporter dye: “JOE”
  - Quencher dye: (none)
  - Color: “Green”
- Click “ok”
- Select both detectors and click “Add >>” to assign the detectors to the plate document
- Select “none” in the drop down menu of “Passive Reference”
- Click “Done”
- Go to the tab “Setup” and “Plate”
- Select all 96 wells of the plate
- Go to the menu point “View” and open the “Well Inspector”
- Select detector “Septin9” and “ACTB”
- Check the Passive Reference setting to be “none” (see Figure 1)
- Click “Close”
- Go to tab “Instrument” to program the cycling conditions as described in Table 7
- Change the following settings:
  - Sample Volume: 30 µl
  - Run Mode: Standard 7500
  - Data Collection: Stage 2, Step 2

- Create a “Thermal Profile” with 3 stages.
- Create a “Stage 2” having 3 steps, and a “Stage 1” and “Stage 3” having one step.
- Enter repetitions, target temperature, and hold time according to Table 7.

Change the “Ramp Rate” according to Table 7

- Set “Data Collection” for “Stage 2, Step 2 (55.5 @ 0:35)”
- Confirm the Thermal Cycler Protocol settings by comparing to Figure 2
- Save the run plate document under an appropriate file name
- A window will open asking for the “Reason for change of entry.” Enter “Setup” and any other comments relevant to the run
- Open the tray
- Place the PCR plate into the frame (position A1 goes to the upper left corner), ensure that the plate fits accurately in the frame. Close the tray.
- Start the run by pressing the “Start” button

Table 7: Thermal Cycler Program (Applied Biosystems 7500 Fast Dx with SDS v1.4)

Program Parameter	Denaturation	Cycling			Holding
Stage	”Stage 1”	”Stage 2”			”Stage 3”
Repetitions	1	45			1
Step	1	1	2	3	1
Target [°C]	94	62	55.5	93	40
Hold [mm:ss]	20:00	00:05	00:35	00:30	00:05
Auto Increment		0	0	0	
Ramp Rate [%]	40	80	80	40	80
Data Collection	Stage 2, Step 2 (55.5 @ 0:35)				

Figure 1: Screenshot from SDS v1.4 software after confirming settings in the 'Well Inspector' window.

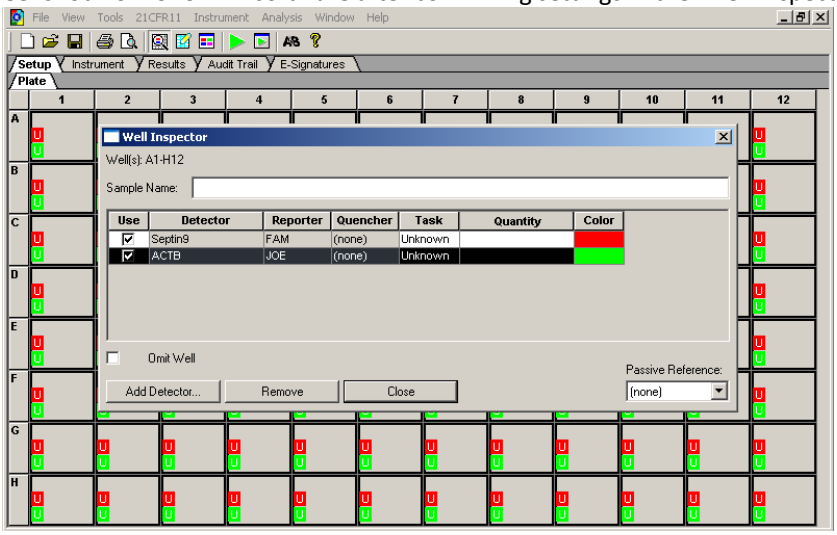
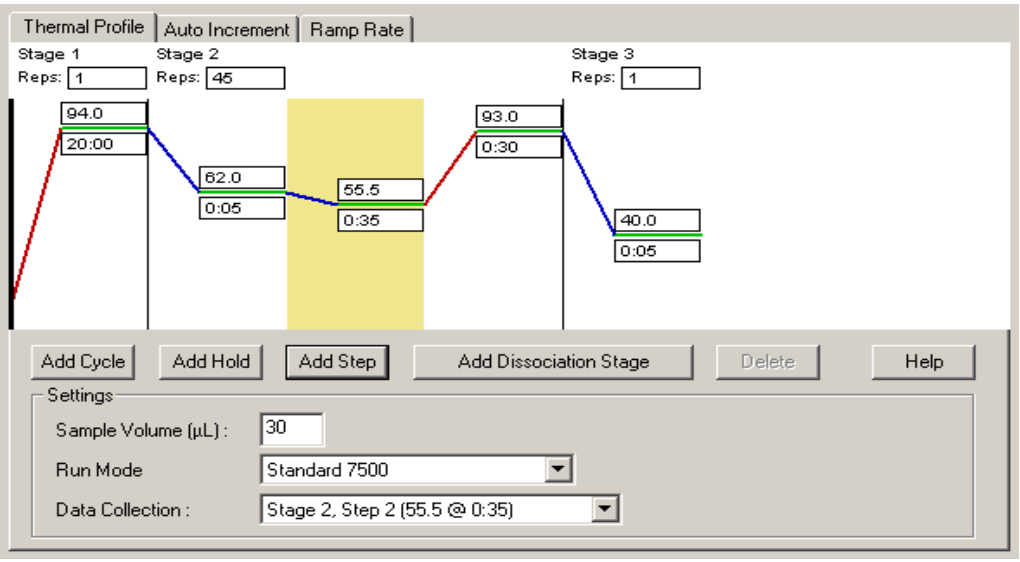
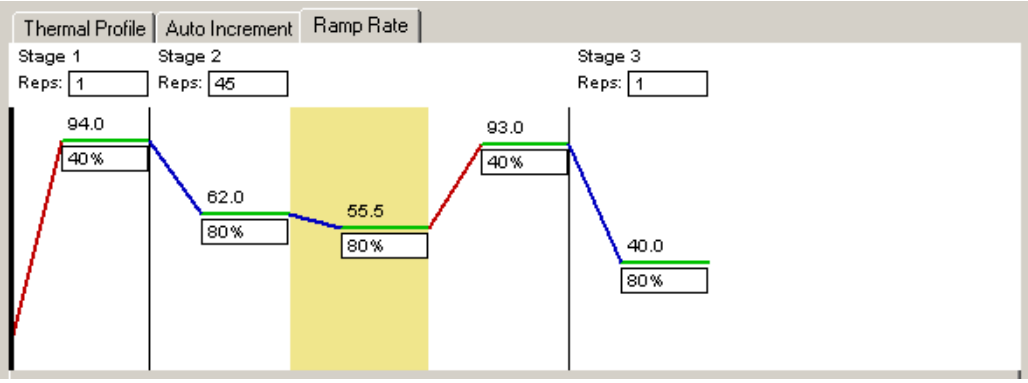
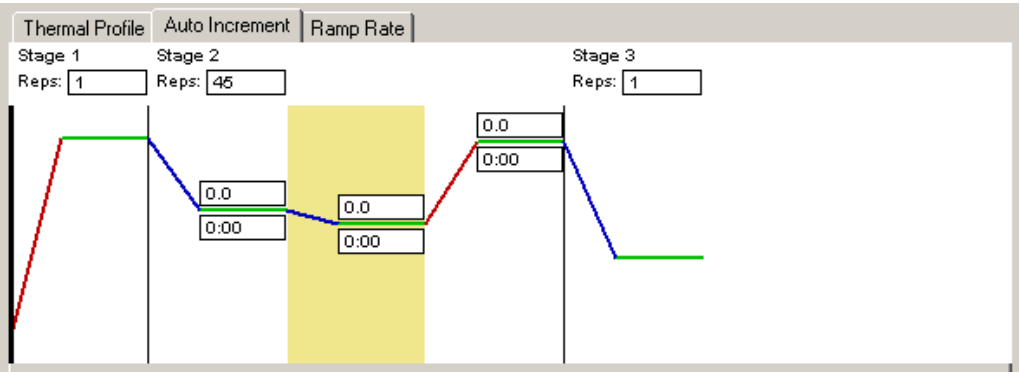


Figure 2: Screenshots from SDS v1.4 software showing correct settings for the cycling program. Temperature, time, repetitions should be displayed as shown in tab 'Thermal Profile' (upper). No changes of the default settings are made in the tab 'Auto Increment' (middle) and should be displayed as shown. Ramping rates should be displayed as shown in the tab 'Ramp rate' (lower). Sample volume, Run Mode and Data Collection are indicated below the cycling scheme in each of the tabs.







9.17. Results Analysis

Note: ANALYZE PCR RUNS WITH SOFTWARE VERSION SDS V1.4 ONLY. Software updates must not be initiated without written instruction from Epigenomics indicating that the Epi proColon test has been validated for use with the new software.

Note: Incomplete runs or runs where an error message occurs must not be analyzed. The run document must contain fluorescence data for 45 cycles.

- After completion of the PCR cycling program click “ok”
- Select the tab “Results”, then select the tab “Amplification Plot“
- Set “Analysis Setting” for Septin 9 detector to be the following:
  - Data: “Delta Rn vs Cycle”
  - Detector: “Septin9”
  - Line color: “Detector Color”
  - Manual Ct, Threshold: “50000” (appears as “5.0e+004”)
  - Manual baseline, Start (cycle): “10”
  - Manual baseline, End (cycle): “22”
- Set “Analysis Setting” for ACTB detector to be the following:
  - Data: “Delta Rn vs Cycle”
  - Detector: “ACTB”
  - Line color: “Detector Color”
  - Manual Ct, Threshold: “25000” (appears as “2.5e+004”)
  - Manual baseline, Start (cycle): “10”
  - Manual baseline, End (cycle): “22”
- Confirm the correct Analysis Settings for each detector by comparing to Figure 3
- Analyze the data by pressing the “Analyze” button  in the top menu
- Save the file by pressing Save Document in the task bar. A window will open asking for the “Reason for change of entry”. Enter “Data analysis post run” and any other comments relevant to the run.
- Septin 9 Ct values and ACTB Ct values are calculated automatically
- Ct values are displayed in the tab “Report”

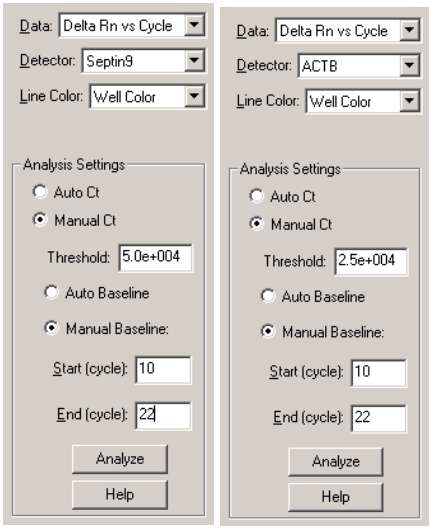


Figure 3: Screenshot from SDS v1.4 software showing correct analysis settings for the Septin 9 detector (left) and ACTB detector (right).

10. Interpretation of Results

Assessment of the Validity of the Run by Epi proColon Controls

Any run of one or more patient sample(s) processed together with the Epi proColon Positive Control and Epi proColon Negative Control is considered VALID, when criteria set forth in Table 8 are met for **ALL THREE (3)** PCR replicates per control.

If either the Epi proColon Positive Control or Epi proColon Negative Control, or both is INVALID, the data for patient specimens processed together with the controls cannot be interpreted. Testing must be repeated for all patient specimens included in this test run.

Table 8: Validity Limits of Epi proColon Control Kit

Result of Control	Determination	Septin 9 Result	ACTB Result
Positive Control VALID	PCR1	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR2	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR3	Ct* ≤ 41.1	Ct* ≤ 29.8
Negative Control VALID	PCR1	No Ct* result ("Undetermined")	Ct* ≤ 37.2
	PCR2		Ct* ≤ 37.2
	PCR3		Ct* ≤ 37.2

*cycle threshold

Assessment of the Validity of a Single PCR of a Patient Specimen

The interpretation of a single PCR is performed according to Table 9. Provided that the result of the internal control assay ACTB indicates sufficient input of bisDNA into the single PCR (ACTB cycle threshold at most 32.1), the result of the Septin 9 PCR defines the result for this single PCR. An ACTB cycle threshold above 32.1 turns the single PCR result to "INVALID PCR".

Table 9: Interpretation of Results for Single PCR

Single PCR Result	Septin 9 Result	ACTB Result
Septin 9 Positive	Ct* < 45**	Ct* ≤ 32.1
Septin 9 Negative	No Ct* result ("Undetermined")	Ct* ≤ 32.1
INVALID	Any result	Ct* > 32.1 Or "Undetermined"

*cycle threshold

**CT values >40 are typically observed for analyte concentrations that are below the Limit of Detection (LoD) of the assay, whereas CT values <40 are observed for concentrations above the Limit of Detection of the assay.

Interpretation of Results for a Patient Specimen

The interpretation of patient test results is found on Table 10. The test result for a patient sample is "POSITIVE", if at least one PCR replicate is a Septin 9 Positive PCR. The test result for a patient specimen is "NEGATIVE", if all three PCR replicates are Septin 9 Negative PCRs. The test result is "INVALID" in all other cases.

Table 10: Interpretation of Epi proColon Test Results

Test Result	Positive Control Negative Control	Single PCR Results
POSITIVE	VALID	At least one Septin 9 Positive PCR[†]
NEGATIVE	VALID	PCR1: Septin 9 Negative PCR PCR2: Septin 9 Negative PCR PCR3: Septin 9 Negative PCR
INVALID	VALID	All other cases[‡]
INVALID	INVALID	n/a

[†] One single PCR result is Septin 9 Positive; the two remaining single PCR results may have any result (INVALID, Septin 9 Negative, or Septin 9 Positive)

[‡] No single PCR result is Septin 9 Positive and at least one single PCR result is INVALID; the remaining single PCR results may be INVALID or Septin 9 Negative.

Note: The assessment of the validity and the interpretation of the Epi proColon test result are performed and recorded using the "Epi proColon Results Form" (IFU 0010) available at the E•Library found at epiprocolon.com.

11. Quality Control

Quality Control procedures are designed to monitor the integrity of reagent performance and ensure the accuracy (validity) of reported test results. As with any control product or feature, patient results should not be reported if control results fail to yield the expected results. Each laboratory should refer to the guidelines and procedures established by their laboratory, appropriate regulatory agencies, or accrediting organizations in accordance to the requirements of each laboratory's Quality Assurance program and accrediting organization.

The external Positive and Negative Controls are contained in the Epi proColon Control Kit (M5-02-003) for use with the Epi proColon Plasma Quick Kit (M5-02-001) and the Epi proColon PCR Kit (M5-02-002). The Internal Control (IC) is a part of the Epi proColon PCR Kit (M5-02-002).

External Controls: The Epi proColon Control Kit (M5-02-003)

One Epi proColon Positive (PC) and one Negative (NC) Controls must be included with every run when testing patient samples using the Epi proColon test kit. These controls monitor the successful execution of the test workflow to ensure the validity of patient test results.

If the Epi proColon Positive and Negative Control test do not yield values within the required validity limits (see Table 8), see Remedial Actions, below.

Internal Control – Duplex β-Actin

For every PCR well, the duplexed detection of bisulfite-converted β-actin (ACTB) DNA serves as an internal control. This co-amplified sequence provides a monitor for sample quality, sample preparation and adequate DNA concentration of the specimen.

The Septin 9 PCR test result is linked to the Ct value of the ACTB PCR (see Table 9). Ct values of the ACTB PCR outside the specified range INVALIDATE the single PCR test result. High Ct values are associated with very low bisDNA or PCR inhibition.

If the Ct values of the ACTB reaction are outside the specified range and INVALIDATE the single PCR result, please refer to Remedial Actions, below.

Remedial Actions

When External Controls (PC, NC) fail to meet the validation criteria, patient test results for the entire run should be considered INVALID and should not be reported. PC and NC and patient samples should be repeated, and if INVALID results are obtained a second time, please contact Epigenomics Customer Support or your local distributor, for assistance.

For an Internal Control that does not meet validation criteria, that single PCR result is INVALID. The patient result should be assessed based on all three PCR results according to Table 10. If the patient result is INVALID based on this assessment, the result for the patient should not be reported.

Each laboratory should perform remedial actions according to their established Quality Assurance program prior to reporting patient test results.

12. Limitations of the Procedure

- This product has been validated for the combination of the Epi proColon Plasma Quick Kit (M5-02-001), the Epi proColon Sensitive PCR Kit (M5-02-002), and the 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.
- The Epi proColon test has been validated for use only with plasma derived from blood collected with Vacutainer® 10 mL K2 EDTA blood collection tubes (Becton Dickinson). Do not perform this test with other clinical specimen types or with other blood collection tubes.
- The 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.
- The Limit of Detection of the assay was determined to be above the assay cutoff.

13. Performance Characteristics

Clinical Sensitivity and Specificity

Three studies have been performed to demonstrate the clinical performance of the Epi proColon test. The first trial compared the performance of the Epi proColon test to colonoscopy. The second compared the performance of the Epi proColon test and a Fecal Immunochemical Test (OC FIT-CHEK®) using colonoscopy as the reference method. The third (ADMIT) compared adherence to CRC screening in a randomized population using the Epi proColon blood test and a FIT test (OC FIT-CHEK®).

Trial One

In a large, prospective multicenter trial (PRESEPT), 7,941 men (45%) and women (55%) ages 50 to 85, who were of average-risk for colorectal cancer were enrolled at 32 clinical sites in the US and Germany (ClinicalTrials.gov, Trial Registration ID: NCT00855348). The average-age of the screening-eligible cohort was 60 years. The study was conducted under the US Multi-Society Task Force Guideline. For this study, 1,544 plasma specimens of 6857 available from the PRESEPT study cohort were tested with Epi proColon at three independent clinical laboratories in the US.

The 1,544 samples included all CRC patients, all patients with advanced adenomas (AA), and a random subset of subjects with small polyps (SP) or no evidence of disease (NED).[8] Selection of SP and NED subjects was performed using a stratified random sampling approach based on the US 2010 census and the intended use population. Although not all the specimens in the PRESEPT study were tested, all relevant subpopulations were sampled and tested to derive the maximum value from the available sample pool. Clinical performance of the Epi proColon test was evaluated in terms of sensitivity for colorectal cancer and specificity in subjects negative for colorectal cancer, as determined by colonoscopy. The results for the study by overall clinical status (CRC and non-CRC) are shown in Table 11, with the sensitivity and specificity outcome in Table 12, along with positive and negative predictive values. Specificity of Epi proColon decreased with increasing age, as shown by increasing positive detection fractions within increasing age groups in Table 13.

Table 11: Result of Epi proColon Testing Sample Type

Sample Status	Negative	Positive	VALID Samples
CRC	14	30	44
Non-CRC	1182	318	1500
Total	1196	348	1544

Table 12: Clinical Performance of Epi proColon

Sample Status	Point Estimate	Lower 95%	Upper 95%
Sensitivity	68.2 %	53.4 %	80.0 %
Specificity	78.8 %	76.7 %	80.8 %
Specificity* (weighted)	79.1 %	77.0 %	81.4 %
Specificity** (weighted)	80.0%	77.9 %	82.1 %
Positive Predictive Value	2.4%	2.0%	3.0%
Negative Predictive Value	99.7%	99.6%	99.8%

* weighting according to US census population ** weighting according to PRESEPT patient disposition

Table 13: Results of Epi proColon Testing by Subject Age

Age	Negative	Positive	Total Samples	Positive Detection Fraction (%)	CI 95%
50 – 54	282	60	342	18	14 - 22%
55 – 59	229	40	269	15	11 - 20%
60 – 64	222	64	286	22	18 - 28%
65 – 69	200	66	266	25	20 - 30%
70 – 74	169	52	221	24	18 - 30%
75+	80	36	116	31	23 - 40%
Total	1182	318	1500	21	19 – 23%

Trial Two

In the second clinical trial, the clinical performance of the Epi proColon test was compared to the OC FIT-CHEK® (Polymedco, Inc.) test in a prospective, multi-center study.[9] The study was designed to collect matched blood and fecal specimens and clinical data from screening guideline-eligible subjects using colonoscopy as the reference method.

Subjects were recruited at 61 clinical sites in the US according to the following scheme:

- Subjects having CRC or a high suspicion of invasive CRC identified during screening colonoscopy were enrolled and provided blood and fecal samples at least 10 days after colonoscopy but prior to surgery or intervention
- Prospectively enrolled subjects provided blood and fecal samples prior to bowel prep for screening colonoscopy

Of 337 subjects enrolled in the study, 36 were excluded due to failure to meet inclusion/exclusion criteria. From the remaining 301 enrolled subjects, there were 101 CRC, 29 AA, 77 SP and 94 NED. Plasma samples were available from all 301 subjects. Fecal samples were not available from 11 subjects (four CRC, two AA, two SP and three NED).

Both, the Epi proColon testing and OC FIT-CHEK testing were performed at an independent US diagnostic laboratory. Test outcome was compared to results obtained from colonoscopy for both methods.

Overall sensitivity and specificity for the Epi proColon test and FIT test for all available samples and on paired samples as compared to the reference colonoscopy are calculated in Tables 14 a, b and c, with a three-way summary in Table 15.

The observed sensitivity for CRC on paired samples was 4.2% higher for the Epi proColon test (Table 14 b, c). The 95% confidence interval (-16.2%; 8.1%) was strictly below the non-inferiority margin of 10% pre-set in the protocol. The sensitivity of the Epi proColon test is statistically non-inferior to the FIT test.

For specificity, the difference between tests was 16.6% in favor of the FIT with a 95% confidence limit (10.6%; 22.9%) around the estimate (Table 14 b, c). This result does not demonstrate non-inferiority for specificity when compared to the non-inferiority margin of 20% pre-set in the protocol.

Table 14a: Epi proColon Sensitivity and Specificity, n = 301 All Plasma Samples

Parameter	Point Estimate	CI 95%
Sensitivity	73.3% (74/101)	63.9 – 80.9%
Specificity	81.5% (163/200)	75.5 – 86.3%

Table 14b: Epi proColon Sensitivity and Specificity, n = 290 Paired Samples

Parameter	Point Estimate	CI 95%
Sensitivity	72.2% (70/97)	62.5 – 80.1%
Specificity	80.8% (156/193)	74.7 – 85.8%

Table 14c: FIT Sensitivity and Specificity, n = 290 Paired Samples (equals number of available fecal samples)

Parameter	Point Estimate	CI 95%
Sensitivity	68.0% (66/97)	58.2 – 76.5%
Specificity	97.4% (188/193)	94.1 – 98.9%

Table 15: Three-Way Comparison of the Epi proColon and FIT Tests, and Colonoscopy (paired samples)

Diagnostic Accuracy Criteria: Standard Colonoscopy						
	Colorectal Cancer (CRC)			**Non-Colorectal Cancer AA, SP, NED**		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
FIT Positive	50	16	66	1	4	5
FIT Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

Trial Three

In a prospective, multicenter clinical trial (ADMIT), consented subjects were randomized to the blood-based Epi proColon test or a stool-based FIT test (OC FIT-CHEK®) with the primary objective of comparing adherence to CRC screening.

There were 413 eligible patients enrolled at two geographically distinct health systems. Of these, 185 of 210 subjects in the FIT arm, and 202 of 203 subjects in the Epi proColon arm were adherent to screening, Table 16. Adherence rates for Epi proColon blood test and FIT were 99.5% (CI, 97.3%-100%) and 88.1% (CI, 83.0%-91.8%), respectively. For Epi proColon, 182 of 202 blood samples obtained valid results, leaving 20 blood tests incomplete due to phlebotomy and laboratory errors. For FIT, 179 of 185 stool samples obtained valid results, leaving six samples with no result. Adherence to screening was calculated based on all samples obtained from patients for testing regardless of validity.

At both trial locations, a behavioral passive control group comprised of 213 subjects (Site 1, 88; Site 2, 125) was offered CRC screening via the institutional standard of care. Of the 88 subjects invited for screening via colonoscopy at Site 1, 11/88 (12.5%) participated. Of the 123 subjects invited for screening with FIT and two via colonoscopy at Site 2, 29 participated with FIT and one with colonoscopy for a total of 30/125 (24.0%). A total of 41/213 subjects participated in screening resulting in an overall passive control adherence rate of 19.2% (CI, 14.5%-25.1%).

Table 16: Adherence to CRC Screening with Epi proColon and FIT

	Epi proColon			**FIT**		
	Site 1	**Site 2**	**Total**	**Site 1**	**Site 2**	**Total**
Adherent	85	117	202	72	113	185
Non-Adherent	1	0	1	12	13	25
Total	86	117	203	84	126	210

The trial design included the secondary objectives of reporting diagnostic yield as well as compliance to colonoscopy for subjects yielding positive results from either test method. There were 182 valid Epi proColon test results, and 179 valid FIT test results. Positivity rates for the Epi proColon blood test and FIT were 16.5% (30/182) and 1.7% (3/179). Subjects with positive results were counseled to undergo colonoscopy. For subjects with a positive Epi proColon result, 17/30 (56.7%) completed colonoscopy; of these cases, 10/17 (58.8%) resulted in actionable findings (polypoid, polyps, adenomas) and 0 (0%) were diagnosed as CRC. For subjects with a positive FIT result, 1/3 (33.3%) completed colonoscopy; of these cases, one (100%) resulted in actionable findings (polyps, adenomas) and none (0%) were diagnosed with CRC.

Table 17: Adherence to Colonoscopy with Epi proColon and FIT

	Epi proColon			FIT		
	Site 1	**Site 2**	**Overall**	**Site 1**	**Site 2**	**Overall**
Positivity Rate	20.5% (14/68)	14.0% (16/114)	16.5% (30/182)	4.4% (3/68)	0% (0/111)	1.7% (3/179)
Schedule Rate	50.0% (7/14)	81.3% (13/16)	67.7 (20/30)	66.7% (2/3)	N/A	66.7% (2/3)
Compliance Rate	35.7%* (5/14)	75.0%** (12/16)	56.7% (17/30)	33.3% (1/3)	N/A	33.3% (1/3)
Adenoma/Polyp Detection Rate	60.0% (3/5)	58.3% (7/12)	58.8% (10/17)	100.0% (1/1)	N/A	100.0% (1/1)

*2 colonoscopies scheduled after study; ** 1 colonoscopy canceled

Analytical Sensitivity - Limit of Detection

The Limit of detection (LoD) of the Epi proColon test was determined at four laboratories according to CLSI EP12 and EP17 guidance documents. All sites utilized the test kit manufactured under final manufacturing conditions. Seven levels of technical samples were tested with a range of methylated DNA from 0 (blank) to 50 pg/mL. LoDs were determined using DNA spiked into plasma and into an artificial matrix of Tris buffer plus BSA. Based on the results of this study, the LoD is 4.7 pg/mL (95% CI 2.5-9.0 pg/mL).

Precision and Accuracy

Accuracy – Internal study

The assay Ct thresholds for the Epi proColon test were established in a study performed using 156 clinical plasma samples (reference method: colonoscopy). The instrumentation was set to 50 cycles in order to establish the Septin 9 and ACTB cycle threshold (Ct) limits on these clinical specimens. From the distributions of these data, it was evident that Ct values larger than 40 for Septin 9 PCR are rare and no Ct-values are observed above a Ct of 45. The vast majority of ACTB measurements range between 26 to 30 Ct. There was a general observation in these clinical specimens of outliers having lower Ct values. For very few specimens the ACTB value came close to Ct of 31. From these data Septin 9 and ACTB Ct limits of 45 and 32.1, respectively, were determined.

Accuracy – Cut-off Verification

A second study was performed using 346 clinical plasma samples. The instrumentation was set at 50 cycles for a subset of 197 plasma samples in order to verify the Septin 9 and ACTB cycle threshold limits of 45 and 32.1, respectively, on these clinical specimens. The distributions of these data confirmed the results from the first study. Therefore, the cycle threshold limits were verified. For these 346 clinical samples, the overall agreement with clinical status was observed at 94.9% (93/98) with a 95% CI from 88.6% – 97.8% for CRC cases, and 84.3% (209/248) with a 95% CI from 79.2% – 88.3% for colonoscopy-verified controls.

Repeatability, Intermediate Precision and Reproducibility

To evaluate repeatability and intermediate precision of the assay, aliquots from 14 clinical sample pools were tested at three testing sites by six operators with three reagent lots using three PCR instruments. Each plasma pool was tested 12 times. Pools 1 - 6 generated from CRC plasma were positive in all 12 tests. For the three pools representing self-declared healthy blood donors, pool 7, pools 8 and pool 9 were each negative in 9 out of 12 tests. For the pools derived by diluting a single CRC plasma aliquot in human plasma, pools 10, 13, and 14 were positive in 11 out of 12 tests, while pools 11 and 12 were positive in all 12 replicates. In total, for 129 out of 132 samples where CRC plasma was tested (pools 1 - 6, pools 10 - 14), the test result was positive leading to 98% (95% CI: 94 - 99%) positive percent agreement with clinical status. Aggregated over the three pools derived from healthy blood donors (pools 7 - 9), the test results for 27 out of 36 samples was negative leading to 75% (95% CI: 59 - 86%) negative percent agreement with clinical status. The total percent agreement estimated from these data is 156/168, i.e. 93% (95% CI: 88 - 96%). There were no differences in the positive and negative percent agreement attributable to sites, operators or kit lots.

In addition, precision and reproducibility analyses were conducted with Ct values generated on the set of 14 sample pools. For precision, the ranges of standard deviation and coefficient of variation (CV) for Septin 9 were 0.4 - 2.1 Ct and 1.1 - 5.5%, respectively. The corresponding ranges for ACTB were 0.2 - 0.4 Ct and 0.8 - 1.7%.

For reproducibility, the ranges of standard deviation and coefficient of variation for Septin 9 were 0.4 - 2.3 Ct and 1.4 - 6.0%, respectively. The corresponding ranges for ACTB were 0.2 - 0.4 Ct and 0.7 - 1.6%.

Cross-Reactivity

To test for sequence cross-reactivity, BLAST alignment searches and electronic PCR analyses were performed against the human genome with the Epi proColon PCR assay (blocker, primers and probe). This analysis showed that he test is specific for amplification of the bisDNA sequence of methylated Septin 9 and therefore showed no cross-reactivity within the human genome.

Three-hundred and sixty four samples from patients with other cancers (prostate, breast, lung) or other diseases (hypertension, hyperlipidemia, diverticulitis, chronic gastritis, or cardiovascular) with no evidence of CRC by colonoscopy, were evaluated to determine cross-reactivity. The Epi proColon test was positive in 72 of 173 patients (42%) with other cancers and positive in 33 of 191 patients (17%) with other diseases. The methylation status of the Septin 9 region was determined for 7 positive patients (3 patients with other cancers, 4 patients with other diseases) by bisulfite sequencing of the PCR product. For all test positives, sequencing confirmed methylation of the Septin 9 PCR target area. This study demonstrates that the Epi proColon test detects the true biological status for these other conditions rather than reporting cross-reactivity.

Interfering Substances

A study was conducted to verify that the presence of interfering substances potentially found in plasma samples have no effect on the test results. The ten (10) most common substances present in human plasma were selected and tested at the highest concentration that would occur in a clinical setting. Albumin (40 mg/mL), Bilirubin (0.2 mg/mL), Cholesterol (5 mg/mL), D-(+) Glucose (10 mg/mL), Hemoglobin (10 mg/mL), K_2EDTA (20 mg/mL), Red Blood Cells (0.4 % v/v), Triglycerides (12 mg/mL), human genomic DNA (100 ng/mL), and Uric Acid (0.235 mg/mL) were tested in 6 plasma specimens each (3 analyte negative, 3 analyte positive). None of the substances interfere with the test when added to the specimen at the listed concentration level.

14. Meaning of Symbols

	Consult Instructions for Use
	Order Number
	*In Vitro* Diagnostic Medical Device
	Lot Number
	Use By Date
	Manufacturer

	Temperature Limits for Storage
	Contains sufficient materials for <n> tests
	Do not reuse
	Negative Control
	Positive Control
	CE mark (made in compliance with 98/79/EC Directive on IVDs)
R x O n l y	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
	Unique Device Identifier

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15. Contact Information

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Find Out More

To learn more about Epigenomics and our products, please visit **epiprocolon.com**, and select the **"Q & A"** tab where you will find answers to commonly asked questions about the Epi proColon test. Please contact us in any of the other following ways:

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Epi proColon® is an FDA-approved blood test for colorectal cancer screening. This test is intended for persons age 50 and older who are unwilling or unable to be screened by recommended methods.

Rx Only

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Colorectal cancer is one of the most preventable and treatable cancers when it's found early.

What is Epi proColon®?

Epi proColon is a blood test for colorectal cancer screening that can be ordered by your doctor. The test is for people who are unwilling or unable to use screening tests recommended in the 2008 United States Preventive Services Task Force (USPSTF) guidelines.[2] About 7 out of 10 patients who have colorectal cancer have a positive Epi proColon test result. About 2 out of 10 people without cancer have a positive Epi proColon test result.

The Epi proColon test is performed by a clinical laboratory. It detects a specific type of DNA called Septin 9. Septin 9 DNA is altered in colorectal cancer tumor cells more often than in normal cells. The tumor cells release altered Septin 9 DNA into the bloodstream. The altered DNA can be detected in a blood sample. Altered Septin 9 DNA is often found in blood samples from people with colorectal cancer.[3, 4]

What is Colorectal Cancer Screening?

Screening is looking for colorectal cancer before there are symptoms. Screening is necessary to find cancer early. When colorectal cancer is found early, successful treatment is more likely. About 9 out of 10 people will survive when it is found early.[1] There are different screening tests to choose from. You and your doctor can work together to find the test that works best for you.

Colorectal Cancer in the United States

- No. 3 cancer
- No. 3 cause of death from cancer
- Only 4 out of 10 people are diagnosed early before it has spread outside the colon

Who Should Be Screened for Colorectal Cancer?

Anyone can get colorectal cancer. Today, 1 out of 3 people are not screened. It is recommended that people of "average-risk" should start screening at age 50.[1] Average-risk means that there is about a 1 in 20 chance of getting colorectal cancer in your lifetime. This risk increases as you age. If you have risk factors for colorectal cancer, you should talk to your doctor about what screening plan is right for you. See page 7 to learn more about risk factors.

Colorectal Cancer Screening Tests

There are two types of screening tests recommended by the United States Preventive Services Task Force (USPSTF) guidelines.

- Invasive imaging tests that may find and prevent cancer.

  These include colonoscopy and flexible sigmoidoscopy. CT colonography is not included in these guidelines.

- Non-Invasive tests that may tell you about your risk for cancer.

  These include high-sensitivity stool tests that detect blood in stool. Stool DNA and blood tests are not included in these guidelines.

It might be hard to decide what test you should have. Before choosing, you should understand the benefits and risks for each test. Talk to your doctor about which test would be best for you.

Is Epi proColon Right for You?

You should talk with your doctor about recommended screening tests so that you can choose the test that is best for you. Recommended methods include colonoscopy and FIT stool tests.

This test might be an option for you if:

- You are age 50 or older
- You are considered average-risk for getting colorectal cancer*
- You are out-of-date with recommended screening tests
- You decline recommended screening tests after talking with your doctor

NOTE: If you are over age 75, you should talk with your doctor about whether getting screened is right for you.

This test is NOT an option for you if:

- You are less than age 50
- You are considered higher-risk for getting colorectal cancer*
- You are up-to-date with recommended screening
- You are willing and able to be screened with recommended screening tests

* More information about risks is found on page 7 of this brochure.

How Do You Get Tested with Epi proColon?

Your blood test can be ordered by your doctor during your routine office visit. Your blood can be drawn at your doctor's office or the blood collection center. You do not have to change your diet or medicine before getting your blood drawn.

What Do You Need To Do?

1. Make an appointment with your doctor to discuss colorectal cancer screening. If you both decide this test is right for you, your doctor will write a lab order for the test.
2. Get your blood drawn. It might take a few days for the laboratory to send your test result to your doctor.
3. Talk with your doctor about your test result.

If your test result is positive, your doctor will recommend a colonoscopy. If your test result is negative, discuss your future screening plan with your doctor.

How Accurate is Epi proColon?

Epi proColon finds cancer in about 7 out of 10 (70%) people with the disease. Epi proColon also has positive results in people who do not have cancer. These are called false-positive results. Positive results were found in about 2 out of 10 (20%) people who do not have cancer.

Epi proColon was studied for accuracy in three studies:

Study One[5]

The first study compared Epi proColon to colonoscopy. The study included 1,544 men and women, age 50 to 85 years who were average-risk for colorectal cancer. The study was conducted at 32 sites in the United States and Germany.

Epi proColon Sensitivity = 68.2%	Epi proColon False-Positive Rate = 20.0%	Negative Predictive Value (NPV) = 99.7%	Positive Predictive Value (PPV) = 2.4%
This means that the blood test was positive in about 7 out of 10 people with colorectal cancer.	This means that the blood test was positive in about 2 out of 10 people who did not have colorectal cancer.	This means that a person with a negative result has a very low chance of having colorectal cancer.	This means a person with a positive test has a 2.4% chance of having colorectal cancer.

People Not Tested in Study One

- **People at higher-risk for colorectal cancer**
- **People with rectal bleeding or fresh blood in the stool**
- **People with a known history of iron deficiency**

Person with a positive result; Person with a positive result with colorectal cancer
PPV = percent chance that a person with a positive test result has colorectal cancer
NPV = percent chance that a person with a negative test result does not have colorectal cancer

Study Two[7]

The second study compared Epi proColon to a Fecal Immunochemical Test (FIT) at 61 sites in the United States. This study included 290 people. Each person gave a blood and stool sample for testing. In this study, both tests found colorectal cancer at all cancer stages. Also, both tests found cancer in different places throughout the colon and rectum.

Epi proColon Test

Epi proColon Sensitivity = 72.2%	Epi proColon False-Postive Rate = 19.2%	Negative Predictive Value (NPV) = 99.8%	Positive Predictive Value (PPV) = 2.7%
This means that the blood test was positive in about 7 out of 10 people with colorectal cancer.	This means that the blood test was positive in about 2 out of 10 people who did not have colorectal cancer.	This means that a person with a negative result has a very low chance of having colorectal cancer.	This means a person with a positive test has a 2.7% chance of having colorectal cancer.

FIT Stool Test

FIT Sensitivity = 68.0%	FIT False-Positive Rate = 2.6%	Negative Predictive Value (NPV) = 99.8%	Positive Predictive Valuie (PPV) = 15.6%
This means that the stool test was positive in about 7 out of 10 people with colorectal cancer.	This means that the stool test was positive in less than 1 out of 10 people who did not have colorectal cancer.	This means that a person with a negative result has a very low chance of having colorectal cancer.	This means a person with a positive test has a 15.6% chance of having colorectal cancer.

Person with a positive result; Person with a positive result with colorectal cancer
PPV = percent chance that a person with a positive test result has colorectal cancer
NPV = percent chance that a person with a negative test result does not have colorectal cancer

NOTE: In Study One, the chance of having colorectal cancer was found to be about 0.7%. This number was used in the formula for NPV and PPV for Study Two.

Study Three[3]

The third study compared the number of people that would take a blood or stool test for colorectal cancer screening. The study included 413 people at two health sites. All people in the study had been offered screening at least two times in the past and were not up-to-date.

Epi proColon	FIT
• **203 people were offered the blood test. 202 completed it (99.5%).** • **30 people had a positive result. Of those, 17 had a colonoscopy and 10 of those had a polyp or adenoma removed**	• **210 people were offered the stool test for colorectal cancer screening. 185 completed it (88.1%)** • **3 people had a positive test result. Of those, 1 had a colonoscopy and had a polyp removed.**

NOTE: More information about these studies can be found at ClinicalTrials.gov. Details for Study One (NCT00855348), Study Two (NCT01580540) and Study Three (NCT02251782) may be found by looking up these study numbers.

What are the Benefits and Risks of Screening with Epi proColon?

Benefits

Getting screened for colorectal cancer is important. When colorectal cancer is found early, cure is more likely.[1] If you are not up-to-date and unwilling or unable to be screened by recommended tests, Epi proColon may be an option for you.

- Epi proColon is a blood test ordered by your doctor
- Your blood may be drawn at your doctor's office or a blood collection center
- No changes in diet or medicine are needed before your blood draw

Risks

- Epi proColon is not intended to replace colorectal screening tests that are recommended by 2008 USPSTF guidelines. These tests include colonoscopy, sigmoidoscopy and high-sensitivity stool blood tests.
- Epi proColon was positive more times in people without colorectal cancer (false-positives) than a stool test (OC FIT-CHEK® Polymedco, Inc.). Both tests were positive equally in people who had colorectal cancer.
- If your Epi proColon test is negative, you should talk with your doctor about what screening tests you should do for the next year and the years thereafter. If after talking with your doctor you still decline recommended tests, Epi proColon is an option for you.
- In clinical trials, the Epi proColon test was given to people only one time. It was not given to people who had been tested with Epi proColon before. It is not known how well the test will perform when used more than one time over a period of years.
- The chance of having a false-positive test result goes up with age. You should talk to your doctor about what a false-positive result might mean for you.
- Positive test results have been found in healthy people. Positive results have also been found in people who have chronic gastritis or lung cancer, and also in pregnant women.[3, 7]

Understanding Your Epi proColon Test Results

What does a Positive Epi proColon blood test result mean?

- A positive blood test means that altered Septin 9 DNA has been found in your blood sample.
- A positive blood test result increases the chance that you may have colorectal cancer. However, it does not mean that you have colorectal cancer.
- You should discuss your test result with your doctor. Your doctor should order a colonoscopy to find out if you have colorectal cancer.

What does a Negative Epi proColon blood test result mean?

- A negative blood test result means that altered Septin 9 DNA was not found in your blood sample.
- A negative test result decreases the chance that you have colorectal cancer.
- Some people with colorectal cancer may have negative test results. Studies show that altered Septin 9 DNA is not found in the blood of every person with colorectal cancer.[3]
- Even if you have a negative result, you should continue to get screened for colorectal cancer on a regular basis. Talk with your doctor about your personal health history. You should consider getting a colonoscopy, sigmoidoscopy, or high-sensitivity stool blood test at your next screening.

More About Risk Factors for Colorectal Cancer

A risk factor is anything that increases your chance for getting cancer. Your personal risk factors like family history, lifestyle and ethnicity will determine when you should start screening.[1]

What are Personal Risk Factors?

- You or a close member of your family (parent, sibling, child) has had colorectal cancer. Your risk is higher when two or more family members have had colorectal cancer. It is also higher if one or more of your family members has colorectal cancer and is less than age 50.
- You have a history of benign polyps in the colon or rectum or related cancers, or have other bowel disease like inflammatory bowel disease (IBD), chronic ulcerative colitis (CUC), or Crohn's disease.
- You have inherited diseases like Lynch syndrome (hereditary non-polyposis colorectal cancer) or FAP (familial adenomatous polyposis).
- You have other inherited diseases include Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner's syndrome, Turcot's (or Crail's) syndrome, Cowden's syndrome, Juvenile Polyposis, Cronkhite-Canada syndrome, Neurofibromatosis, or Familial Hyperplastic Polyposis.

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epi pro colon®

Real-Time PCR
Single-Day Test Protocol
Flexible Workflow

Rx Only

epigenomics

Detecting Cancer In Blood.

Intended Use

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

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

Contraindications

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

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

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

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

Warnings, Precautions and Limitations

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

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

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

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

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

The rate of false positive Epi proColon results increases with age. Test results should be interpreted with caution in elderly patients. See Performance Characteristics in Section 13.[1]

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

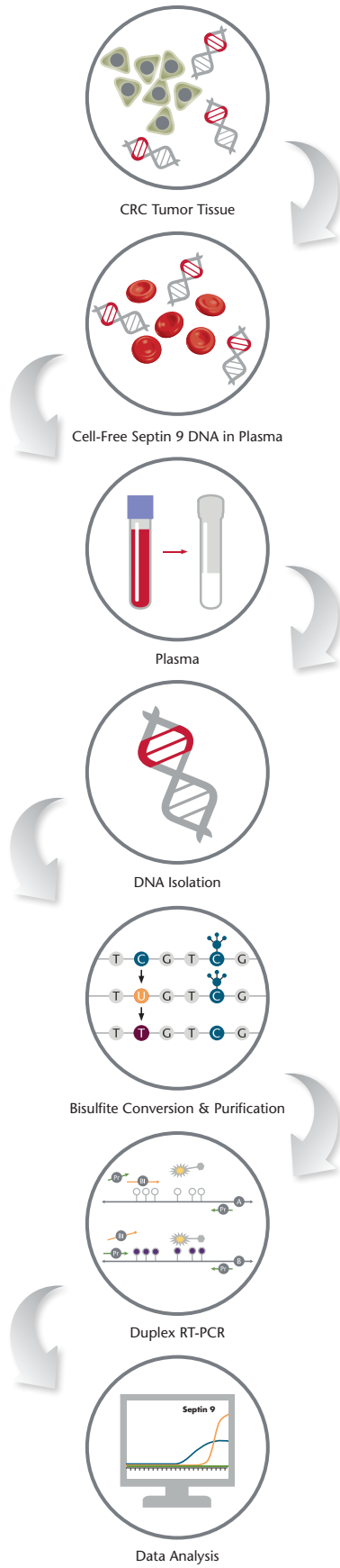
Positive test results have been observed in healthy subjects and in patients diagnosed with chronic gastritis, lung cancer and in pregnant women.[1,2]

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

What is Epi proColon®?

Epi proColon is a molecular test that detects methylated Septin 9 DNA in blood. DNA methylation of the *SEPT9* gene is increased in colorectal cancer. Methylated Septin 9 tumor DNA is shed into the bloodstream and displays a unique methylation pattern that is detectable in plasma by Real-Time PCR.[3,4]

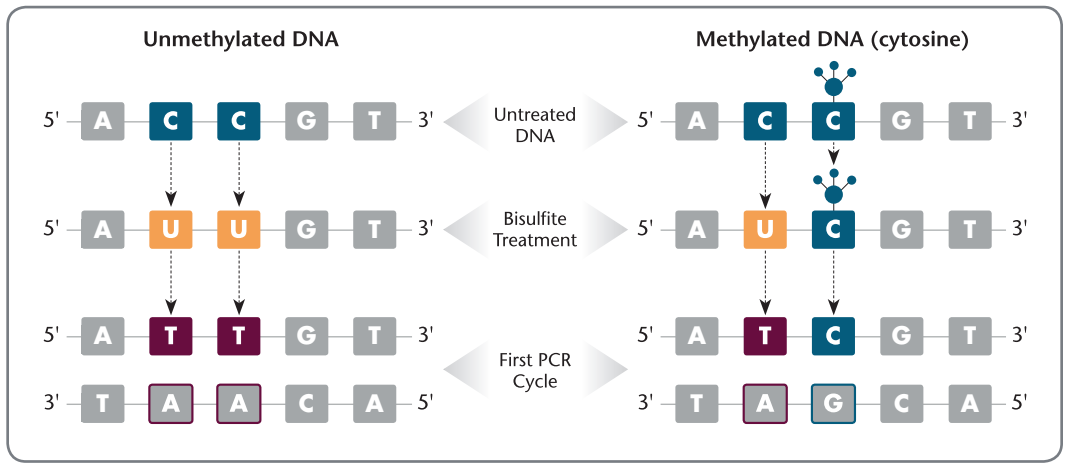
RIGHT:
The Epi proColon test is for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument.



Detecting Methylated Septin 9 DNA

Cytosine residues in the *v2* region of the *SEPT9* gene may become methylated in colorectal cancer tissues. When DNA isolated from plasma samples is treated with a high concentration of bisulfite, unmethylated cytosines are converted to uracil while methylated cytosines remain unchanged (Figure 1). As a consequence of treatment, the DNA sequence is altered based on methylation status and can be analyzed by Real-Time PCR amplification (Figure 1).[3, 4]

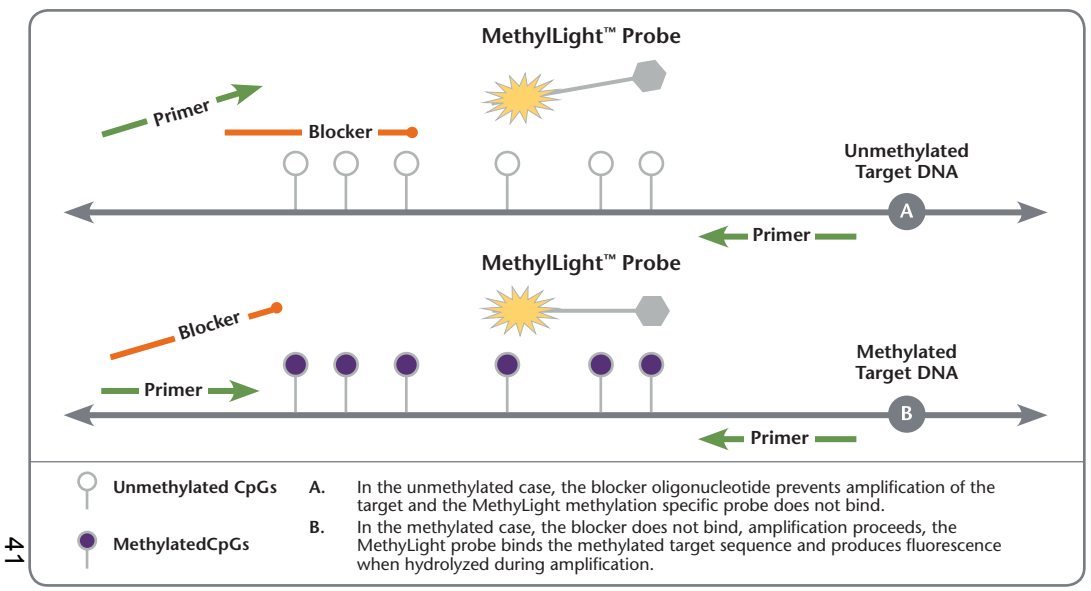
FIGURE 1: Detecting DNA Methylation



HeavyMethyl® Core Technology

The Epigenomics' HeavyMethyl core technology combines the use of primers that amplify the target biomarker regardless of methylation status, with a blocking oligonucleotide to suppress the amplification of unmethylated DNA, and a methylation-specific probe to detect the amplified methylated sequence (Figure 2). The proprietary HeavyMethyl core technology enables detection of low copy number tumor DNA in a background of non-tumor DNA in plasma.[3, 4]

FIGURE 2: HeavyMethyl® Real-Time PCR



Epi proColon® Features & Benefits

Complete Test Kit Offers Convenience and Efficiency

- DNA Extraction and Bisulfite Conversion Reagents
- PCR Reagents
- External Positive and Negative Controls

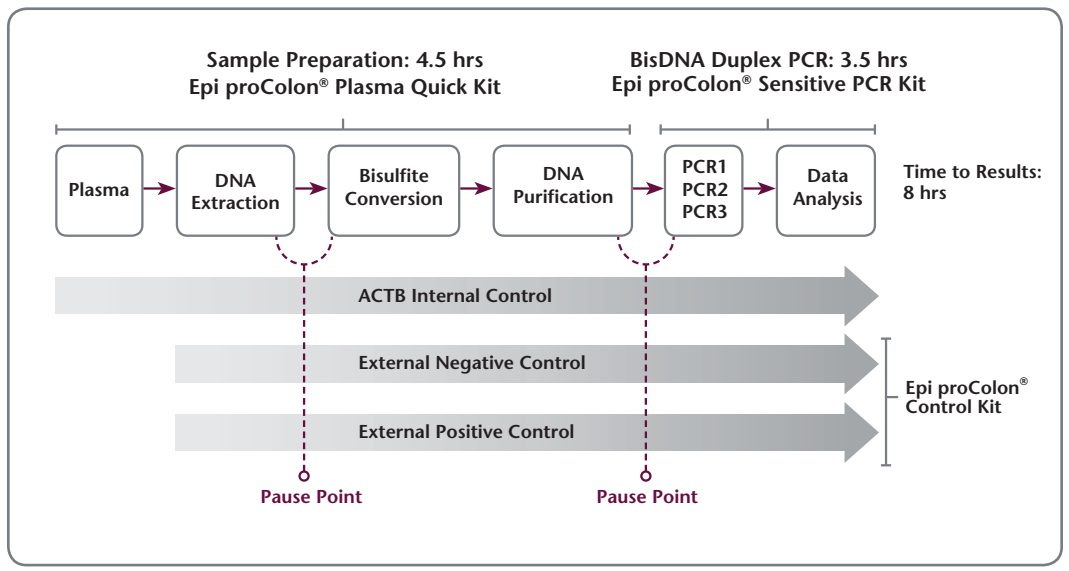
Quality Control Verifies Workflow and Validity

- **Internal Process Control:**
  Co-amplified internal control monitors sample quality, sample preparation and adequate DNA concentration
- **External Controls:**
  Positive and Negative Controls performed identically to patient samples monitor successful workflow and ensure validity of patient test results

Simple Real-Time PCR Test—Basic Molecular Lab Technology

- Familiar PCR technology
- Flexible workflow adapts to staff workload requirements (Figure 3)
- Single day protocol with TTR usually < 8 hours

FIGURE 3: The Epi proColon Test and Workflow



Clinical Trials Overview

Trial One[5]

The first study compared the accuracy of Epi proColon to colonoscopy in 1,544 samples from men and women, 50–85 years of age who were of average-risk for CRC.

Sensitivity (95% CI)	**Specificity** (95% CI)	**People Not Tested in the Study**
68.2% (53.4–80.0)	80.0% (77.9–82.1)*	• People considered at higher-risk for developing CRC • People with rectal bleeding, fresh blood in the stool, or with a known history of iron deficiency
Negative Predictive Value (NPV) (95% CI)	**Positive Predictive Value (PPV) (95% CI)**	
99.7% (99.6–99.8)	2.4% (2.0–3.0)	

* Weighted to the Study One Population.

Trial Two[6]

The second study compared the accuracy of Epi proColon to a Fecal Immunochemical Test (FIT) using matched blood and stool samples from 290 people. Epi proColon was found to be statistically non-inferior to FIT with respect to sensitivity but not specificity.

Epi proColon (95% CI) (n=290)	
Sensitivity 72.2% (62.5–80.1) Specificity 80.8% (74.7–85.8)	NPV 99.8% (99.7–99.8) PPV 2.7% (2.0–3.7)

FIT (95% CI) (n=290)	
Sensitivity 68.0% (58.2–76.5) Specificity 97.4% (94.1–98.9)	NPV 99.8 (99.7–99.8) PPV 15.6 (7.2–30.8)

Both Tests

- Identified similar numbers of patients with CRC
- Identified CRC in all cancer stages and throughout the colon and rectum

NOTE: Assumes a prevalence of 0.7% based on Study One for Positive and Negative Predictive Values with 95% CI. Predictive values inform how likely disease is given the test result. PPV indicates how likely disease is given a positive test result. NPV indicates how likely absence of disease is given a negative test result.

Trial Three[1]

The third study compared participation in CRC screening among 413 people who were offered either a stool test or a blood test. All people in the study had at least two screening recommendations in the past and were not up-to-date with their screening.

Epi proColon

- 203 people were offered the blood test for CRC screening and 202 completed it (99.5%)
- 30 people had a positive Epi proColon test result; of those, 10 out of the 17 people who completed a colonoscopy had a polyp or adenoma removed

FIT

- 210 people were offered the stool test for CRC screening and 185 completed it (88.1%)
- 3 people had a positive FIT test result; of those, 1 completed a colonoscopy and had a polyp removed

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Clinical Trials Summary

Trial One[5]

In a large, prospective multicenter clinical trial, 7,941 women and men ages 50 to 85, who were of average-risk for colorectal cancer were enrolled at 32 clinical sites in the US and Germany. The clinical performance of the Epi proColon test was evaluated in 1,544 of the trial participants using colonoscopy as the reference standard (Table 1). The study included all patients with cancer (all stages) or advanced adenomas and a stratified random sample of patients with small polyps, and patients with no evidence of disease (NED), (Tables 2 and 3). The Epi proColon test showed sensitivity, specificity, positive and negative predictive values of 68.2%, 78.8%, 2.4% and 99.7% respectively (Table 1).

TABLE 1: Epi proColon performance with colonoscopy as the reference standard

Sensitivity = 68.2% (95% CI, 53.4–80.0)
Specificity = 78.8% (95% CI, 76.7–80.8)
Specificity* = 80.0% (95% CI, 77.9–82.1)
Negative Predictive Value (NPV) = 99.7% (CI, 99.6–99.8)
Positive Predictive Value (PPV) = 2.4% (CI, 2.0–3.0)
Positive Test Rate = 22.5% (95% CI, 20.6–24.6)

		Epi proColon		
		Negative	Positive	Total
Colonoscopy	CRC	14	30	44
	Non-CRC	1182	318	1500
	Total	1196	348	1544

* Weighted to the Study One population.

PPV = percent probability that a person with a positive test result has CRC
NPV = percent probability that a person with a negative test result does not have CRC

TABLE 2: Epi proColon clinical trial results for different patient groups

Classification	Positives (Total)
NED (No Evidence of Disease)	97 (444)
Polyps	87 (435)
Advanced Adenomas	134 (621)

TABLE 3: Epi proColon clinical trial results for colorectal cancer (CRC) Stages

CRC Stage	Epi proColon % (Positives/Total)
Stage I	41.1% (7/17)
Stage II	83.3% (10/12)
Stage III	80.0% (8/10)
Stage IV	100.0% (5/5)
Total Cancers	68.2% (30/44)

Trial Two[6]

The performance of Epi proColon test and a fecal immunochemical test (OC FIT-CHEK®) were determined in a multicenter trial at 61 US sites (Table 4). Blood, fecal specimens and clinical data were collected, and performance compared to colonoscopy as the reference method. The study enrolled subjects who: 1) had CRC or a high suspicion of invasive CRC identified by screening colonoscopy—blood and fecal samples were collected at least 10 days after colonoscopy but prior to surgery or intervention; or 2) provided blood and fecal samples prior to bowel prep for screening colonoscopy.

TABLE 4: Performance characteristics for CRC detection of Epi proColon and OC FIT-CHEK test

Method	Sensitivity (95% CI)	Specificity (95% CI)
Epi proColon n=301	73.3% (74/101) (63.9–80.9)	81.5% (163/200) (75.5–86.3)
Epi proColon* n=290	72.2% (70/97) (62.5–80.1)	80.8% (156/193) (74.7–85.8)
OC FIT-CHEK* n=290	68.0% (66/97) (58.2–76.5)	97.4% (188/193) (94.1–98.9)

* Based on paired samples

NOTE: The observed sensitivity for CRC on paired samples was 4.2% (95% CI, -8.1–16.2) higher for Epi proColon (Table 5). The sensitivity of Epi proColon is statistically non-inferior to the OC FIT-CHEK test. For specificity, the difference between the two tests was 16.6% (95% CI, 10.6 –22.9) in favor of the FIT test and does not demonstrate non-inferiority.

Plasma samples were available from 301 eligible people (101 CRC, 29 advanced adenomas (AA), 77 small polyps (SP), 94 with no evidence of disease (NED). Fecal samples were not available from 11 of these subjects (4 CRC, 2 AA, 2 SP and 3 NED). Sensitivity and specificity for the Epi proColon test (73.3%, 81.5%) and OC FIT-CHEK tests (68.0%, 97.4%) were in the expected range (Table 4). Based on these results, sensitivity of the Epi proColon test was statistically non-inferior to that of OC FIT-CHEK while specificity was not. The Epi proColon test also detected cancers at the earliest clinical stages in this trial (Table 5).

TABLE 5: Epi proColon and OC FIT-CHEK clinical trial results for colorectal cancer (CRC) stages

CRC Stage	Epi proColon % (Positives/Total)	FIT % (Positives/Total)
Stage 0	100.0% (2/2)	0.0% (0/2)
Stage I	61.5% (16/26)	65.4% (17/26)
Stage II	80.0% (16/20)	80.0% (16/20)
Stage III	65.2% (15/23)	82.6% (19/23)
Stage IV	92.3% (12/13)	58.3% (7/12)
Unknown	76.5% (13/17)	50.0% (7/14)
Total Cancers	73.3% (74/101)	68.0% (66/97)

TABLE 6: Three-way comparison of Epi proColon, OC FIT-CHEK and colonoscopy results

	Diagnostic Accuracy Criteria: Standard Colonoscopy					
	Colorectal Cancer			Non-Colorectal Cancer AA, SP, NED		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
OC FIT-CHEK Positive	50	16	66	1	4	5
OC FIT-CHEK Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

In a three way comparison, the tests were compared with colonoscopy as the reference (Table 6). In this study, combining the two tests would increase sensitivity to 89%, with little impact on specificity.

Trial Three[1]

In a third, prospective multicenter clinical trial (ADMIT), 413 eligible men and women from two health systems were randomized to the Epi proColon blood test or a FIT test (OC FIT-CHEK®). All people enrolled in the trial had at least two screening recommendations in the past and were not up-to-date. The primary and secondary study objectives were to compare adherence to CRC screening (Table 7) and compliance to colonoscopy for people who had positive results from either method (Table 8).

Adherence rates for Epi proColon blood test and FIT were 99.5% (95% CI, 97.3–100) and 88.1% (95% CI, 83.0–91.8), respectively, an observed difference in adherence of 11.4% (95% CI, 6.9–15.9). For Epi proColon, 182 of 202 blood samples obtained valid results, leaving 20 blood tests incomplete due to phlebotomy and laboratory errors. For FIT, 179 of 185 stool samples obtained valid results, leaving six samples with no result. Adherence to screening was calculated based on all samples obtained from patients for testing regardless of validity. People with positive results were counseled to undergo colonoscopy. For people with a positive Epi proColon result, 17/30 (56.7%) completed colonoscopy; of these cases, 10/17 (58.8%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed as CRC. For people with a positive FIT result, 1/3 (33.3%) completed colonoscopy; of these cases, 1 (100%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed with CRC.

TABLE 7: Adherence to CRC Screening with Epi proColon and FIT

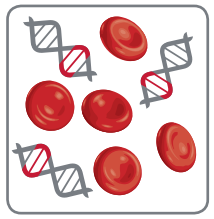
	Epi ProColon			FIT		
	Site 1	Site 2	Total	Site 1	Site 2	Total
Adherent	85	117	202	72	113	185
Non-Adherent	1	0	1	12	13	25
Total	86	117	203	84	126	210

TABLE 8: Adherence to Colonoscopy with Epi proColon and FIT

	Epi ProColon			FIT		
	Site 1	Site 2	Overall	Site 1	Site 2	Overall
Positivity Rate	20.5% (14/68)	14.0% (16/114)	16.5% (30/182)	4.4% (3/68)	0% (0/111)	1.7% (30/179)
Schedule Rate	50.0% (7/14)	81.3% (13/16)	67.7% (20/30)	66.7% (2/3)	N/A	66.7% (2/3)
Compliance Rate	35.7%* (5/14)	75.0%** (12/16)	56.7% (17/30)	33.3% (1/3)	N/A	33.3% (1/3)
Adenoma/Polyp Detection Rate	60.0% (3/5)	58.3% (7/12)	58.8% (10/17)	100% (1/1)	N/A	100% (1/1)

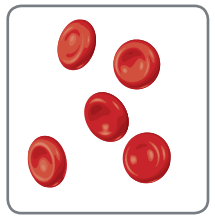
*2 colonoscopies scheduled after study; ** 1 colonoscopy canceled

Understanding Epi proColon Results



A **POSITIVE BLOOD TEST RESULT** indicates that methylated Septin 9 DNA has been detected in the plasma sample tested. Methylated Septin 9 has been associated with the occurrence of colorectal cancer.[3] Because the Epi proColon test is not a confirmatory test for the presence of colorectal cancer, patients with positive Epi proColon test results should be referred for diagnostic colonoscopy.

NOTE: Positive results have been observed in healthy patients and clinically diagnosed patients with chronic gastritis, lung cancer and in pregnant women.[1,2] Because a colonoscopy procedure examines the interior lining of the colon and rectum, CRC is unlikely when no abnormal findings are discovered during this procedure.



A **NEGATIVE BLOOD TEST RESULT** indicates the absence of methylated Septin 9 DNA in the plasma sample tested. Because a negative test result is not confirmatory for the absence of colorectal cancer, people should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

NOTE: Studies show that methylated Septin 9 DNA is not present in plasma from all patients with colorectal cancer and therefore, a negative test result does not guarantee absence of cancer.[1] Detection of CRC is dependent on the amount of circulating tumor DNA in the plasma specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.[1,3]

Epi proColon Test Kit

30 Patient Plasma Samples • 2 Controls • 96 Well Format

Provided	Required
Epi proColon Plasma Quick Kit (M5-02-001)	BD Vacutainer® K2EDTA Blood Collection Tubes†
Epi proColon PCR Kit (M5-02-002)	
Epi proColon Control Kit (M5-02-003)	

Required Instrumentation
Life Technologies™ Instrument and Software Specification†† Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with SDS v1.4 21, CFR Part 11 Module

† This product has been validated ONLY for use with BD Vacutainer® K2EDTA collection tubes; other reagents and consumables as required for Real-Time PCR are detailed in the Epi proColon Test Kit Instructions for Use (IFU 0008).[1]

†† This product has been validated ONLY for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument and software system.

Refer to the Epi proColon Test Instructions for Use (IFU 0008) for more information regarding user requirements.[1]

CPT Code Information

81401, Molecular pathology procedure, Level 2, *SEPT9* (Septin 9) (e.g., colon cancer), methylation analysis.

epi pro colon®

Find Out More

To learn more about **Epi proColon**, please visit **epiprocolon.com**, and select the **"Q & A"** tab where you will find answers to commonly asked questions.

Email	Support.US@epiprocolon.com
Online	epiprocolon.com
US Toll-free	844 537 4669 (844 5 EPI NOW)
Corporate Phone	240 747 7002
Corporate Fax	240 747 7052

REFERENCES

1 Epi proColon Instructions for Use (IFU 0008) and Epigenomics data on file, P130001.
2 Warren, D. et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med. 2011, 133(9).
3 deVos T et al. Circulating methylated *SEPT9* DNA in plasma is a biomarker for colorectal cancer. Clin Chem. 2009, 55(7):1337-1346.
4 Lofton-Day C et al. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem. 2008, 54(2):414-423.
5 Potter N et al. Validation of a Real-Time PCR-based qualitative assay for the detection of methylated *SEPT9* DNA in human plasma. Clin Chem. 2014, 60(9):1183-1191.
6 Johnson D et al. Plasma Septin9 versus fecal immunochemical testing for colorectal cancer screening: a prospective multicenter study. PLOS ONE. 2014, 9(6):1-8. E98238.
7 United States Preventive Services Task Force (USPSTF). Screening for colorectal cancer recommendations statement. Oct, 2008.

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Corporate Phone	914 739 5400
Corporate Fax	914 739 5890

epi pro colon®

Epi proColon® is an FDA-approved blood test for colorectal cancer screening for patients who are unwilling or unable to be screened by recommended methods.

Rx Only

epigenomics

Detecting Cancer In Blood.

Intended Use

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

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

Contraindications

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

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

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

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

Warnings, Precautions and Limitations

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

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

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

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

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

The rate of false positive Epi proColon results increases with age. Test results should be interpreted with caution in elderly patients. See Performance Characteristics in Section 13.[1]

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

Positive test results have been observed in healthy subjects and in patients diagnosed with chronic gastritis, lung cancer and in pregnant women.[1,2]

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

Screening Recommendations

The US Preventive Services Task Force, the American Cancer Society and other medical groups recommend colorectal cancer screening for men and women beginning at the age of 50. There are a number of screening tests to choose from including colonoscopy and fecal tests. Epi proColon provides an additional option to consider for colorectal cancer screening for your patients who have received counseling and have a history of not completing screening by colonoscopy and fecal tests.

epi pro colon®

What You Should Know About Epi proColon,® and the Septin 9 DNA Biomarker

Epi proColon is a molecular test that detects methylated Septin 9 DNA in blood.[1,3]

DNA methylation of the *SEPT9* gene is increased in colorectal cancer.[1,3,4]

Methylated Septin 9 DNA can be found in tumor DNA that has been shed into the bloodstream from proximal and distal colon and rectal sites, making it a differential biomarker for the early detection of colorectal cancer. [1,3,4]

NOTE: Studies show that methylated Septin 9 DNA is not present in plasma from all patients with colorectal cancer and therefore, a negative test result does not guarantee absence of cancer.[1] Detection of CRC is dependent on the amount of circulating tumor DNA in the plasma specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.[1,3]

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epi pro colon®

Patient Testing with Epi proColon®

Your patient's blood sample may be drawn in your office laboratory or other local or US clinical laboratories as designated by your patient's healthcare plan.

About Getting Tested

The test does not require pretest dietary or medication restrictions before blood is drawn.

Within a few days, you receive your patient's test result.

Share the Epi proColon test results with your patient, and together, decide if there is any additional follow-up necessary.

Patients with positive Epi proColon test results should be referred for diagnostic colonoscopy.

About the Benefits

If after counseling, your patient still declines CRC screening by colonoscopy and stool tests, Epi proColon is an approved test choice to consider for your patient.

A blood test is a routine and patient-accepted method of testing.

Epi proColon detects methylated Septin 9 DNA that is associated with colorectal cancer.[1,3,4]

There are no pre-test restrictions before drawing blood for Epi proColon.

When found early, CRC is usually curable.[5]

Choice and preference are key factors that influence patient behavior.[6,7]

NOTE: Epi proColon has been validated ONLY with the BD Vacutainer® K2EDTA Blood Collection Tube. See Precautions and Contraindications in this brochure; refer to Epi proColon Instructions for Use (IFU 0008) for more information on results interpretation.

Clinical Trials Overview

Trial One[8]

The first study compared the accuracy of Epi proColon to colonoscopy in 1,544 samples from men and women, 50–85 years of age who were of average-risk for CRC.

Sensitivity (95% CI)	**Specificity** (95% CI)	**People Not Tested in the Study**
68.2% (53.4–80.0)	80.0% (77.9–82.1)*	• People considered at higher-risk for developing CRC • People with rectal bleeding, fresh blood in the stool, or with a known history of iron deficiency
Negative Predictive Value (NPV) (95% CI)	**Positive Predictive Value (PPV) (95% CI)**	
99.7% (99.6–99.8)	2.4% (2.0–3.0)	

* Weighted to the Study One Population.

Trial Two[9]

The second study compared the accuracy of Epi proColon to a Fecal Immunochemical Test (FIT) using matched blood and stool samples from 290 people. Epi proColon was found to be statistically non-inferior to FIT with respect to sensitivity but not specificity.

Epi proColon (95% CI) (n=290)	
Sensitivity 72.2% (62.5–80.1) Specificity 80.8% (74.7–85.8)	NPV 99.8% (99.7–99.8) PPV 2.7% (2.0–3.7)

FIT (95% CI) (n=290)	
Sensitivity 68.0% (58.2–76.5) Specificity 97.4% (94.1–98.9)	NPV 99.8% (99.7–99.8) PPV 15.6% (7.2–30.8)

Both Tests

- Identified similar numbers of patients with CRC
- Identified CRC in all cancer stages and throughout the colon and rectum

NOTE: Assumes a prevalence of 0.7% based on Study One for Positive and Negative Predictive Values with 95% CI. Predictive values inform how likely disease is given the test result. PPV indicates how likely disease is given a positive test result. NPV indicates how likely absence of disease is given a negative test result.

Trial Three[1]

The third study compared participation in CRC screening among 413 people who were offered either a stool test or a blood test. All people in the study had at least two screening recommendations in the past and were not up-to-date with their screening.

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- 203 people were offered the blood test for CRC screening and 202 completed it (99.5%)
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FIT

- 210 people were offered the stool test for CRC screening and 185 completed it (88.1%)
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Clinical Trials Summary

Trial One[8]

In a large, prospective multicenter clinical trial, 7,941 women and men ages 50 to 85, who were of average-risk for colorectal cancer were enrolled at 32 clinical sites in the US and Germany. The clinical performance of the Epi proColon test was evaluated in 1,544 of the trial participants using colonoscopy as the reference standard (Table 1). The study included all patients with cancer (all stages) or advanced adenomas and a stratified random sample of patients with small polyps, and patients with no evidence of disease (NED), (Tables 2 and 3). The Epi proColon test showed sensitivity, specificity, positive and negative predictive values of 68.2%, 78.8%, 2.4% and 99.7% respectively (Table 1).

TABLE 1: Epi proColon performance with colonoscopy as the reference standard

Sensitivity = 68.2% (95% CI, 53.4–80.0)
Specificity = 78.8% (95% CI, 76.7–80.8)
Specificity* = 80.0% (95% CI, 77.9–82.1)
Negative Predictive Value (NPV) = 99.7% (CI, 99.6–99.8)
Positive Predictive Value (PPV) = 2.4% (CI, 2.0–3.0)
Positive Test Rate = 22.5% (95% CI, 20.6–24.6)

		Epi proColon		
		Negative	**Positive**	**Total**
Colonoscopy	**CRC**	14	30	44
	Non-CRC	1182	318	1500
	Total	1196	348	1544

* Weighted to the Study One population.

PPV = percent probability that a person with a positive test result has CRC
NPV = percent probability that a person with a negative test result does not have CRC

TABLE 2: Epi proColon clinical trial results for different patient groups

Classification	**Positives** (Total)
NED (No Evidence of Disease)	97 (444)
Polyps	87 (435)
Advanced Adenomas	134 (621)

TABLE 3: Epi proColon clinical trial results for colorectal cancer (CRC) Stages

CRC Stage	**Epi proColon** % (Positives/Total)
Stage I	41.1% (7/17)
Stage II	83.3% (10/12)
Stage III	80.0% (8/10)
Stage IV	100.0% (5/5)
Total Cancers	68.2% (30/44)

Trial Two[9]

The performance of Epi proColon test and a fecal immunochemical test (OC FIT-CHEK®) were determined in a multicenter trial at 61 US sites (Table 4). Blood, fecal specimens and clinical data were collected, and performance compared to colonoscopy as the reference method. The study enrolled subjects who: 1) had CRC or a high suspicion of invasive CRC identified by screening colonoscopy—blood and fecal samples were collected at least 10 days after colonoscopy but prior to surgery or intervention; or 2) provided blood and fecal samples prior to bowel prep for screening colonoscopy.

TABLE 4: Performance characteristics for CRC detection of Epi proColon and OC FIT-CHEK test

Method	Sensitivity (95% CI)	Specificity (95% CI)
Epi proColon n=301	73.3% (74/101) (63.9–80.9)	81.5% (163/200) (75.5–86.3)
Epi proColon* n=290	72.2% (70/97) (62.5–80.1)	80.8% (156/193) (74.7–85.8)
OC FIT-CHEK* n=290	68.0% (66/97) (58.2–76.5)	97.4% (188/193) (94.1–98.9)

* Based on paired samples

NOTE: The observed sensitivity for CRC on paired samples was 4.2% (95% CI, -8.1–16.2) higher for Epi proColon (Table 5). The sensitivity of Epi proColon is statistically non-inferior to the OC FIT-CHEK test. For specificity, the difference between the two tests was 16.6% (95% CI, 10.6 –22.9) in favor of the FIT test and does not demonstrate non-inferiority.

TABLE 5: Epi proColon and OC FIT-CHEK clinical trial results for colorectal cancer (CRC) stages

CRC Stage	Epi proColon % (Positives/Total)	FIT % (Positives/Total)
Stage 0	100.0% (2/2)	0.0% (0/2)
Stage I	61.5% (16/26)	65.4% (17/26)
Stage II	80.0% (16/20)	80.0% (16/20)
Stage III	65.2% (15/23)	82.6% (19/23)
Stage IV	92.3% (12/13)	58.3% (7/12)
Unknown	76.5% (13/17)	50.0% (7/14)
Total Cancers	73.3% (74/101)	68.0% (66/97)

Plasma samples were available from 301 eligible people (101 CRC, 29 advanced adenomas (AA), 77 small polyps (SP), 94 with no evidence of disease (NED). Fecal samples were not available from 11 of these subjects (4 CRC, 2 AA, 2 SP and 3 NED). Sensitivity and specificity for the Epi proColon test (73.3%, 81.5%) and OC FIT-CHEK tests (68.0%, 97.4%) were in the expected range (Table 4). Based on these results, sensitivity of the Epi proColon test was statistically non-inferior to that of OC FIT-CHEK while specificity was not. The Epi proColon test also detected cancers at the earliest clinical stages in this trial (Table 5).

TABLE 6: Three-way comparison of Epi proColon, OC FIT-CHEK and colonoscopy results

	Diagnostic Accuracy Criteria: Standard Colonoscopy					
	Colorectal Cancer			Non-Colorectal Cancer AA, SP, NED		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
OC FIT-CHEK Positive	50	16	66	1	4	5
OC FIT-CHEK Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

In a three way comparison, the tests were compared with colonoscopy as the reference (Table 6). In this study, combining the two tests would increase sensitivity to 89%, with little impact on specificity.

Trial Three[1]

In a third, prospective multicenter clinical trial (ADMIT), 413 eligible men and women from two health systems were randomized to the Epi proColon blood test or a FIT test (OC FIT-CHEK®). All people enrolled in the trial had at least two screening recommendations in the past and were not up-to-date. The primary and secondary study objectives were to compare adherence to CRC screening (Table 7) and compliance to colonoscopy for people who had positive results from either method (Table 8).

Adherence rates for Epi proColon blood test and FIT were 99.5% (95% CI, 97.3–100) and 88.1% (95% CI, 83.0–91.8), respectively, an observed difference in adherence of 11.4% (95% CI, 6.9–15.9). For Epi proColon, 182 of 202 blood samples obtained valid results, leaving 20 blood tests incomplete due to phlebotomy and laboratory errors. For FIT, 179 of 185 stool samples obtained valid results, leaving six samples with no result. Adherence to screening was calculated based on all samples obtained from patients for testing regardless of validity. People with positive results were counseled to undergo colonoscopy. For people with a positive Epi proColon result, 17/30 (56.7%) completed colonoscopy; of these cases, 10/17 (58.8%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed as CRC. For people with a positive FIT result, 1/3 (33.3%) completed colonoscopy; of these cases, 1 (100%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed with CRC

TABLE 7: Adherence to CRC Screening with Epi proColon and FIT

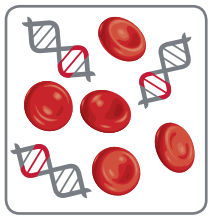
	Epi ProColon			FIT		
	Site 1	Site 2	Total	Site 1	Site 2	Total
Adherent	85	117	202	72	113	185
Non-Adherent	1	0	1	12	13	25
Total	86	117	203	84	126	210

TABLE 8: Adherence to Colonoscopy with Epi proColon and FIT

	Epi ProColon			FIT		
	Site 1	Site 2	Overall	Site 1	Site 2	Overall
Positivity Rate	20.5% (14/68)	14.0% (16/114)	16.5% (30/182)	4.4% (3/68)	0% (0/111)	1.7% (30/179)
Schedule Rate	50.0% (7/14)	81.3% (13/16)	67.7% (20/30)	66.7% (2/3)	N/A	66.7% (2/3)
Compliance Rate	35.7%* (5/14)	75.0%** (12/16)	56.7% (17/30)	33.3% (1/3)	N/A	33.3% (1/3)
Adenoma/Polyp Detection Rate	60.0% (3/5)	58.3% (7/12)	58.8% (10/17)	100% (1/1)	N/A	100% (1/1)

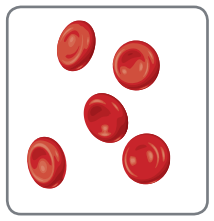
*2 colonoscopies scheduled after study; ** 1 colonoscopy canceled

Understanding Epi proColon Results



A **POSITIVE BLOOD TEST RESULT** indicates that methylated Septin 9 DNA has been detected in the plasma sample tested. Methylated Septin 9 has been associated with the occurrence of colorectal cancer.[3] Because the Epi proColon test is not a confirmatory test for the presence of colorectal cancer, patients with positive Epi proColon test results should be referred for diagnostic colonoscopy.

NOTE: Positive results have been observed in healthy patients and clinically diagnosed patients with chronic gastritis, lung cancer and in pregnant women.[1,2] Because a colonoscopy procedure examines the interior lining of the colon and rectum, CRC is unlikely when no abnormal findings are discovered during this procedure.



A **NEGATIVE BLOOD TEST RESULT** indicates the absence of methylated Septin 9 DNA in the plasma sample tested. Because a negative test result is not confirmatory for the absence of colorectal cancer, people should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

NOTE: Studies show that methylated Septin 9 DNA is not present in plasma from all patients with colorectal cancer and therefore, a negative test result does not guarantee absence of cancer.[1] Detection of CRC is dependent on the amount of circulating tumor DNA in the plasma specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.[1,3]

CPT Code Information

81401, Molecular pathology procedure, Level 2, *SEPT9* (Septin 9) (e.g., colon cancer), methylation analysis.

epi pro colon®

Find Out More

To learn more about **Epi proColon**, please visit **epiprocolon.com**, and select the **"Q & A"** tab where you will find answers to commonly asked questions.

Email	Support.US@epiprocolon.com
Online	epiprocolon.com
US Toll-free	844 537 4669 (844 5 EPI NOW)
Corporate Phone	240 747 7002
Corporate Fax	240 747 7052

REFERENCES

1 Epi proColon Instructions for Use (IFU 0008) and Epigenomics data on file, P130001.
2 Warren J et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med. 2011, 133 (9):1-9.
3 deVos T et al. Circulating methylated *SEPT9* DNA in plasma is a biomarker for colorectal cancer. Clin Chem. 2009, 55(7):1337-1346.
4 Lofton-Day C et al. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem. 2008, 54(2):414-423.
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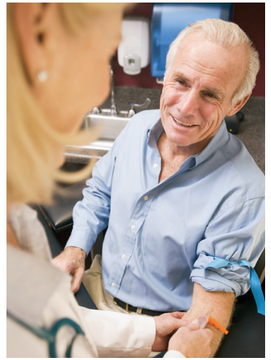
6 Inadomi J et al. Adherence to colorectal cancer screening, a randomized clinical trial of competing strategies. Arch Intern Med. 2012, 172(7): 575-582. doi:10.1001/archinternmed.2012.332.
7 O'Farrell C et al. Physician-patient colorectal cancer screening discussions by physicians' screening rates. JABFM. 2013, 25(6)771-781. doi: 10.3122/jabfm.2012.06.110279
8 Potter N et. al. Validation of a Real-Time PCR-based qualitative assay for the detection of methylated *SEPT9* DNA in human plasma. Clin Chem. 2014, 60(9):1183-1191.
9 Johnson D et al. Plasma Septin9 versus fecal immunochemical testing for colorectal cancer screening: a prospective multicenter study. PLOS ONE. 2014, 9(6):1-8. E98238.
10 United States Preventive Services Task Force (USPSTF). Screening for colorectal cancer recommendations statement. Oct 2008.

How Do My Patients Benefit?



For your patients who have been counseled and offered but declined screening by recommended methods, Epi proColon® is another test choice that may be considered for CRC screening.

- Simple, routine blood test
- Methylated Septin 9 DNA is associated with colorectal cancer[2]
- Providing test choices and considering patient preferences have been cited as key factors that influence patient behavior

Using Epi proColon

- Your patient's blood sample may be drawn at your office laboratory or other local or US clinical laboratories as designated by your patient's healthcare plan.
- Epi proColon has been validated for use ONLY with the BD Vacutainer® K2EDTA blood collection tube.
- Epi proColon is not intended to replace colorectal cancer screening by colonoscopy, sigmoidoscopy or high-sensitivity fecal tests.

When CRC is detected early, cure may still be possible.

A good prognosis is more likely with early diagnosis.

FIND OUT MORE @ epigenomics.com

Intended Use

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

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

Contraindications

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

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

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

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

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FDA APPROVED BLOOD TEST FOR COLORECTAL CANCER SCREENING

epi pro colon®

What is Epi proColon®?

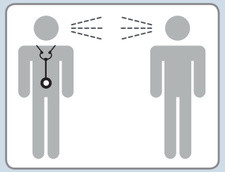
Epi proColon is a blood test for colorectal cancer screening for patients who are unwilling or unable to be screened by recommended methods.[1] The test detects methylated Septin 9 DNA, a differential blood biomarker that is methylated in colorectal cancer. Methylated Septin 9 tumor DNA shed into the bloodstream is detectable by Real-Time PCR.

Rx Only

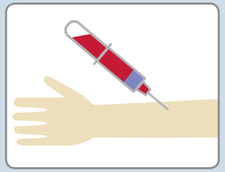
epigenomics
DETECTING CANCER IN BLOOD

How Do My Patients Get Tested?

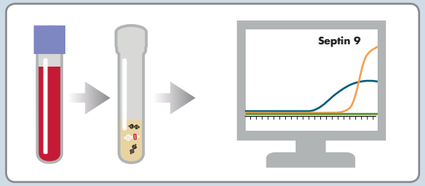
1 Provider Patient Counseling



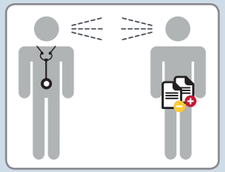
2 Routine Blood Draw



3 Plasma-based testing for Real-Time PCR



4 Patient Management



No pretest dietary or medication restrictions before blood draw.

Testing may take a few days to be completed.

Patients with positive results should be referred for diagnostic colonoscopy.

Clinical Trial Overview

Trial One[3]

The first study compared the accuracy of Epi proColon to colonoscopy in 1,544 samples from men and women, 50–85 years of age who were of average-risk for CRC.

Sensitivity (95% CI)	Specificity (95% CI)
68.2% (53.4–80.0)	80.0% (77.9–82.1)*

Negative Predictive Value (NPV) (95% CI)	Positive Predictive Value (PPV) (95% CI)
99.7% (99.6–99.8)	2.4% (2.0–3.0)

People Not Tested in the Study

- People considered at higher-risk for developing CRC
- People with rectal bleeding, fresh blood in the stool, or with a known history of iron deficiency

* Weighted to the Study One Population.

Trial Two[4]

The second study compared the accuracy of Epi proColon to a Fecal Immunochemical Test (FIT) using matched blood and stool samples from 290 people. Epi proColon was found to be statistically non-inferior to FIT with respect to sensitivity but not specificity.

Epi proColon (95% CI) (n=290)	
Sensitivity 72.2% (62.5–80.1) Specificity 80.8% (74.7–85.8)	NPV 99.8% (99.7–99.8) PPV 2.7% (2.0–3.7)

FIT (95% CI) (n=290)	
Sensitivity 68.0% (58.2–76.5) Specificity 97.4% (94.1–98.9)	NPV 99.8% (99.7–99.8) PPV 15.6% (7.2–30.8)

Both Tests

- Identified similar numbers of patients with CRC
- Identified CRC in all cancer stages and throughout the colon and rectum

NOTE: Assumes a prevalence of 0.7% based on Study One for Positive and Negative Predictive Values with 95% CI. Predictive values inform how likely disease is given the test result. PPV indicates how likely disease is given a positive test result. NPV indicates how likely absence of disease is given a negative test result.

Trial Three[5]

The third study compared participation in CRC screening between people who were offered either a stool test or a blood test. All people in the study had at least two screening recommendations in the past and were not up to date.

Epi proColon

- 203 people were offered the blood test for CRC screening and 202 completed it (99.5%)
- 30 people had a positive Epi proColon test result; of those, 10 out of the 17 people who completed a colonoscopy had a polyp or adenoma removed

FIT

- 210 people were offered the stool test for CRC screening and 185 completed it (88.1%)
- 3 people had a positive FIT test result; of those, 1 completed a colonoscopy and had a polyp removed

REFERENCES

1 United States Preventive Services Task Force (USPSTF). Screening for colorectal cancer recommendations statement. Oct 2008.

2 deVos T et al. Circulating methylated *SEPT9* DNA in plasma is a biomarker for colorectal cancer. Clin Chem. 2009, 55(7):1337-1346.

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