Real-Time PCR
Single-Day Test Protocol
Flexible Workflow

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What is Epi proColon® 2.0 CE?

Epi proColon 2.0 CE is a molecular test that detects methylated SEPT9 DNA in blood. DNA methylation of the SEPT9 gene is increased in colorectal cancer. Methylated SEPT9 tumor DNA is shed into the bloodstream and displays a unique methylation pattern that is detectable in plasma by Real-Time PCR.¹⁻³

LEFT:
The Epi proColon 2.0 CE test is for use with the Applied Biosystems 7500 Fast Dx and Roche LightCycler 480 Real-Time PCR instrument.¹
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 1)
  • Single day protocol with time to results usually < 8 hours

FIGURE 1: The Epi proColon Test Workflow

Note: The Epi proColon Plasma Quick Kit (M5-02-001) and the Epi BiSKit (M7-01-001) are interchangeable for DNA extraction and bisulfite conversion.
Detecting Methylated SEPT9 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 2). As a consequence of treatment, the DNA sequence is altered based on methylation status and can be analyzed by Real-Time PCR amplification (Figure 2).²,³

**FIGURE 2: Detecting DNA Methylation**

![Diagram showing the process of detecting DNA methylation.](image)

**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 3). The proprietary HeavyMethyl core technology enables detection of low copy number tumor DNA in a background of non-tumor DNA in plasma.²,³

**FIGURE 3: HeavyMethyl Real-Time PCR**

![Diagram showing HeavyMethyl Real-Time PCR.](image)

A. In the unmethylated case, the blocker oligonucleotide prevents amplification of the target and the MethyLight methylation specific probe does not bind.

B. In the methylated case, the blocker does not bind, amplification proceeds, the MethyLight probe binds the methylated target sequence and produces fluorescence when hydrolyzed during amplification.
About Epi proColon 2.0 CE

The Epi proColon 2.0 CE test is a qualitative in vitro diagnostic test for the detection of methylated SEPT9 DNA in EDTA plasma derived from patients 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 SEPT9 DNA target.

The Epi proColon 2.0 CE test is indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC. Patients with a positive Epi proColon 2.0 CE test result should be referred for diagnostic colonoscopy. The Epi proColon 2.0 CE test results should be used in combination with physician’s assessment and individual risk factors in guiding patient management.

The Epi proColon 2.0 CE 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.

The Epi proColon 2.0 CE 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.

Clinical Performance and Adherence Overview

Clinical Performance

From an average-risk screening population, prospectively collected clinical samples from 149 patients with no evidence of disease (NED) were enrolled to evaluate the clinical performance of Epi proColon 2.0 CE. Additionally, in a case-control design, 197 clinical samples from 99 colonoscopy-verified negative NED patients and 98 histologically-confirmed colorectal carcinoma patients (all CRC stages) were collected and evaluated.

<table>
<thead>
<tr>
<th></th>
<th>Screening Cohort</th>
<th>Case-Control Cohort</th>
<th>CRC Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Results</td>
<td>149</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>Epi proColon 2.0 CE Positive</td>
<td>1</td>
<td>3</td>
<td>79</td>
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<tr>
<td>Epi proColon 2.0 CE Negative</td>
<td>148</td>
<td>96</td>
<td>19</td>
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<tr>
<td>Specificity</td>
<td>99,3%</td>
<td>96,9%</td>
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</tr>
<tr>
<td></td>
<td>(95,0% CI, 96,3−100,0)</td>
<td>(95,0% CI, 91,5−99,0)</td>
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<tr>
<td>Sensitivity</td>
<td>N/A</td>
<td>N/A</td>
<td>80,6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95,0% CI, 71,7−87,2)</td>
</tr>
<tr>
<td>NPV^</td>
<td>99,9%</td>
<td>99,9%</td>
<td>N/A</td>
</tr>
<tr>
<td>PPV^</td>
<td>28,9%</td>
<td>11,9%</td>
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</table>

NPV and PPV were calculated with a presumed prevalence of 0,5% for CRC in the average-risk population
PPV (Positive Predictive Value) = percent probability that a person with a positive test result has CRC
NPV (Negative Predictive Value) = percent probability that a person with a negative test result does not have CRC
Adherence

In a CRC screening adherence study, 172 people eligible for CRC screening were enrolled and advised to undergo screening by colonoscopy. People unwilling to be screened by colonoscopy were subsequently offered non-invasive blood and stool test options for screening. Of the 109 (63%) people refusing screening colonoscopy, 90 (83%) chose the blood test and 16 (15%), the stool test. This study highlights the importance of offering a non-invasive blood test alternative to increase the acceptance of CRC screening in non-adherent patients, Figure 4.

FIGURE 4: Adherence to colorectal cancer screening

Phase 1: Initial Screening

Chose Colonoscopy

37% (63)

Refused Colonoscopy

63% (109)

Refused Screening

3% (3)

172 screening age-eligible individuals enrolled

97% (106) chose the non-invasive test option

Phase 2: Preferences for Those Refusing Colonoscopy

Chose a Stool Test

15% (16)

Chose a Blood Test

83% (90)
Results Interpretation

A POSITIVE BLOOD TEST RESULT indicates that methylated SEPT9 DNA has been detected in the plasma sample tested. Methylated SEPT9 DNA has been associated with the occurrence of colorectal cancer.² Because the Epi proColon 2.0 CE test is not a confirmatory test for the presence of colorectal cancer, patients with positive Epi proColon 2.0 CE test results should be referred for diagnostic colonoscopy.

A NEGATIVE BLOOD TEST RESULT indicates the absence of methylated SEPT9 DNA in the plasma sample tested. Because a negative test result is not confirmatory for the absence of colorectal cancer, persons should be advised to continue participating in colorectal cancer screening.

NOTE: Patients without a diagnosed CRC but with documented chronic conditions, comorbidity or on medications were tested to determine potential effects on Epi proColon 2.0 CE results and no significant impacts were detected; Positive results have been observed in patients with chronic gastritis, esophagitis and non-rheumatoid arthritis, and lung, breast and prostate cancers; Positive test results have been found in pregnant women; Test results should be interpreted by a healthcare professional.

Epi proColon Test Kit

30 Patient Plasma Samples • 2 Controls • 96 Well Format

<table>
<thead>
<tr>
<th>Provided</th>
<th>Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi proColon Plasma Quick Kit (M5-02-001) or Epi BiSKit™ (M7-01-001)</td>
<td>BD Vacutainer® K2EDTA or Sarstedt S-Monovette® 9.0 mL K3E Blood Collection Tubes†</td>
</tr>
<tr>
<td>Epi proColon PCR Kit (M5-02-002)</td>
<td>Alternately, Sarstedt S-Monovette® 8.5 mL CPDA Blood Collection Tubes</td>
</tr>
<tr>
<td>Epi proColon Control Kit (M5-02-003)</td>
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</table>

Required Instrumentation

Life Technologies™ Instrument and Software Specification††

Applied Biosystems® 7500 Fast Dx or Roche LightCycler 480 Real-Time PCR Instrument with appropriate software version††

† Other reagents and consumables required for Real-Time PCR are detailed in the Epi proColon 2.0 CE Test Kit Instructions for Use (IFU 0009).¹

†† This product has been validated for use with the Applied Biosystems 7500 Fast Dx and the Roche LightCycler 480 Real-Time PCR instrument and software system. Refer to the Epi proColon 2.0 CE Test Instructions for Use (IFU 0009) for description of the appropriate SDS software version.¹

Refer to the Epi proColon 2.0 CE Test Instructions for Use (IFU 0009) for more information regarding user requirements.¹
Find Out More

To learn more about Epi proColon 2.0 CE, please visit epiprocolon.com/en/laboratories where you will find answers to commonly asked questions. Please contact us in any of the other following ways:

Email Support@epigenomics.com
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Phone +49 30 24345 222

REFERENCES

1. Epi proColon 2.0 CE Instructions for Use (IFU 0009) and Epigenomics data on file.