

# Septin 9 (*SEPT9*) Methylated DNA Detection by Real-Time PCR

## *DETECTION OF CIRCULATING METHYLATED SEPTIN 9 DNA FROM BLOOD PLASMA FOR COLORECTAL CANCER SCREENING*

### Test Highlights

- Blood-based assay intended for colorectal cancer screening.
- Outperforms most high-sensitivity fecal occult blood tests without the unpleasantness of stool specimen collection.
- Individuals with detectable *SEPT9* methylated DNA may have colorectal cancer and should be referred for further evaluation.

### Disease Overview

- In the United States, colorectal cancer (CRC) is the third most common form of cancer; individuals have a 6% lifetime risk of developing this disease.
- The American Cancer Society predicted that in 2011 there would be over 140,000 new cases of CRC in the United States, and 50,000 deaths would be attributed to the disease.
- The five-year survival rate of the disease is highly dependent on when the disease is diagnosed; early-stage disease has a 91% survival rate, whereas highly advanced disease has only an 11% five-year survival rate.
- Only one-third of CRC is diagnosed at an early stage, due to the lack of disease symptoms.
- It is recommended that individuals without a family history of the disease begin screening for CRC at the age of 50.
- Less than one-half of individuals in the United States age 50 and older are currently following the recommended screening guidelines.

### Clinical Background

- The presence of *SEPT9* methylated DNA in blood is associated with CRC.
- *SEPT9* methylated DNA has been measured in over 3,000 clinical specimens. The overall performance of the assay based on case-control studies performed in other laboratories has been determined to be 60% to 70% sensitivity and 89% specificity.
- The septin 9 methylated DNA biomarker was evaluated in a large, prospective screening study called PRESEPT involving nearly 8,000 subjects scheduled to have a colonoscopy. Results of the study are as follows:
  - 7,941 screening-eligible subjects of average risk were enrolled in the study at 32 sites in the United States and Germany.
  - 53 cases of unsuspected colorectal cancer in patients were identified by the Septin 9 assay, with over two-thirds being diagnosed as stage I or stage II.
  - In this study, the overall sensitivity in this asymptomatic screening population was 67% with 88% percent specificity.
  - In this study, the negative predictive value of the test was estimated to be 99.7%.
- A *SEPT9* methylated DNA test with improved sensitivity has been developed by ARUP Laboratories.

- Using technical specimens (various concentrations of methylated DNA spiked in normal human plasma), the test was shown to outperform the method used in the PRESEPT study with regard to sensitivity.
- In a case-controlled study of 144 subjects, the test was shown to detect 90% of colorectal cancers.
- In this study, the test had a negative predictive value of 99.94%.

### Indications for Ordering

- This test may be ordered for any individual for whom screening for CRC is recommended.
- This test is not indicated for use in patients deemed to be at above-average risk for CRC (e.g., a patient with a family history of colorectal cancer, a patient with HNPCC, etc.). It is also not indicated for patients who have required previous polyp removal or who have a history of previous CRC.

### Additional Ordering Notes

- Blood specimen should be centrifuged at 1350xg two times for 12 minutes at the time of sample collection. The first centrifugation should occur within four hours of collection, followed by a second centrifugation of the isolated plasma and transfer into a clean freezing vial. Specimens must immediately be frozen at minus 80 degrees centigrade.
- Plasma specimens must be stored and shipped frozen.

### Interpretation

- A positive test result indicates the possible presence of CRC and warrants a recommendation for follow-up evaluation.
- While this test is highly sensitive and specific, not all individuals with CRC will have a positive test result. Therefore, individuals with a negative test result should follow usual colorectal cancer-screening guidelines.

### Limitations

The sensitivity of the assay for detecting stage I colorectal cancer is lower than the overall sensitivity for detecting later-stage CRC, based on studies performed at ARUP and other laboratories.

## Methodology

- DNA is isolated from plasma and treated with a bisulfite reagent.
- Real-time PCR is used to preferentially detect the methylated form of septin 9.
- Each specimen is analyzed in a triplicate PCR reaction in order to maximize sensitivity.
- Assay performance:
  - Sensitivity (all stages of CRC): 90% (95% confidence interval: 77.4% to 96.3%).
  - Sensitivity (early stages of CRC): 87% (95% confidence interval: 71.1% to 95.1%).
  - Specificity: 88% (95% confidence interval: 79.6% to 93.7%).
- These values were established by a case-controlled validation at ARUP Laboratories. The specificity of this assay is comparable to similar studies reported in literature. However, the sensitivity of the test exceeds reported values.

## References

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2. deVos T, et al. Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer. *Clin Chem.* 2009;55(7):1337–46.
3. Church TR, et al. Prospective clinical validation of an assay for methylated SEPT9 DNA in human plasma as a colorectal cancer screening tool in average risk men and women 50 years and older. *Digestive Disease Week Conference.* 2010: LB-711d.
4. Levin B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology.* 2008;134(5):1570–95.

## Test Information

2003243

Septin 9 (SEPT9), Methylated DNA Detection by Real-Time PCR

For specific collection, transport, and testing information, refer to the ARUP website at [www.aruplab.com](http://www.aruplab.com).

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at [www.arupconsult.com](http://www.arupconsult.com).

## AUTHOR

Karen Heichman, PhD