ST57

Epi proColon®, Septin 9 Gene Methylation Detection Assay as a Screening Tool for Colorectal Cancer

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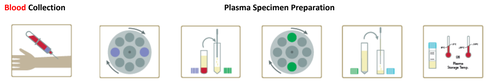
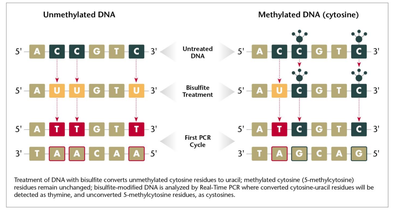
Background

The Epi proColon® test is a qualitative in vitro diagnostic method 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 CRC. This 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 history of not completing CRC screening.

Methods

DNA is isolated from plasma and treated with bisulfite. Real-time PCR is performed on the ABI 7500 Fast Dx to detect the methylated form of Septin 9 DNA. DNA from CRC specimens were used to evaluate accuracy, repeatability and reproducibility of the assay.





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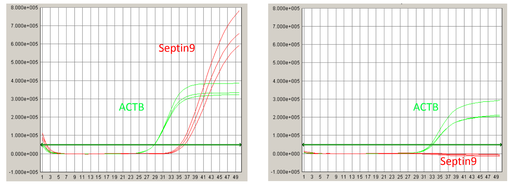
Results

Of the specimens tested during validation, 10 specimens with known CRC clinical status and positive Septin 9 status had analysis results that were 100% concordant when tested at two different clinical sites; 10 specimens with no CRC clinical status and negative Septin 9 status had analysis results that were 75% concordant. The false positive rate from 130 data points was 13.25%, which was lower than the manufacturer's 20% rate. Repeatability (intra-assay precision) was 100% concordant for Septin 9 positive specimens and 88.9% for Septin 9 negative specimens; reproducibility (Inter-assay precision) was 100% concordant for Septin 9 positive specimens and 88.9% for Septin 9 negative specimens.

The Epi proColon® test has been offered as a clinical test in LabCorp based on the successful performance features in the validation data. Of the 3047 specimens tested, 73.94% were negative and 23.57% were positive. Among the positive specimens, 67.78% specimens had one positive result among the triplicate runs, 19.11% had two positive results and 13.11% had three positive results. Results could not be obtained in 2.49% specimens due to low amounts of DNA recovered from the plasmas. The positive detection rate was similar to the manufacturer's rate during assay clinical validation and there was no gender difference. A subset of 350 specimens was from patients <50 years, the positive rate from this group was 16.29% with females at 11.35% and males at 19.61%. The positive rate for patients ≥50 years but <75 was 22.39% (2238 specimens) with females at 19.86% and males at 25.74%. For patients ≥75 years, the overall positive rate was 34.86% (459 specimens) with no significant difference between males (35.08%) and females (34.86%). False positive rate increases with age. Patients with a positive Epi proColon® test result should be referred for diagnostic colonoscopy.

Typical Amplification Plots of Positive and Negative Control



Result of Control	Determination	Septin9 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

Test Result	Positive Control Negative Control	Single PCR Results
Positive	VALID	At least one Septin9 Positive PCR†
Negative	VALID	PCR1: Septin9 Negative PCR
		PCR2: Septin9 Negative PCR
		PCR3: Septin9 Negative PCR
INVALID	VALID	All other cases‡
INVALID	INVALID	n/a

† One single PCR result is Septin9 Positive; the two remaining single PCR results may have any result (INVALID, Septin9 Negative, or Septin9 Positive)
‡ No single PCR result is Septin9 Positive and at least one single PCR result is INVALID; the remaining single PCR results may be INVALID or Septin9 Negative.

Repeatability

Repeatability (intra-assay precision) was 100% concordant for Septin 9 positive specimens and 88.9% for Septin 9 negative specimens.

-3 replicates of 3 wild type pools of 5 samples each and 3 pools of 5 samples each with known methylation in the SEPT9 gene. Pools were utilized as the package insert requires 3 replicates per sample.
-3 clinically positive samples were 100% concordant
-Clinically negative samples NEG 2E and NEG 3A each had Septin9 signal in 1 out of 3 reactions and resulted as positive, 7/9=77.8% concordance among negative samples.
-Overall concordance 16/18 = 88.9%

Reproducibility

reproducibility (Inter-assay precision) was100% concordant for Septin 9 positive specimens and 88.9% for Septin 9 negative specimens.

-3 replicates run on 3 separate assay runs for 3 wild type
pools of 5 samples each and 3 pools of 5 samples each with known methylation in the SEPT9 gene. One of these runs was performed by a second technologist.
-3 clinically positive samples were 100% concordant
Clinically negative samples NEG 2E and NEG 3A each had 1 Septin9 replicate that was positive in Inter 1 (Septin9 signal in 1 out of 3 reactions)
-Concordance among negative specimens: 7/9=77.8
-Overall concordance 16/18 = 88.9%

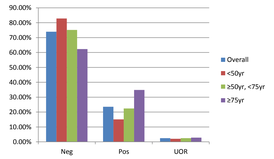
Accuracy

10 specimens with known CRC clinical status and positive Septin 9 status had analysis results that were 100% concordant when tested at two different clinical sites; 10 specimens with no CRC clinical status and negative Septin 9 status had analysis results that were 75% concordant. The false positive rate from 130 data points was 13.25%, which was lower than the manufacturer's 20% rate.

Clinical Specimen Results

	Overall	<50yr	≥50yr, <75yr	≥75yr
Neg	73.94%	82.84%	75.11%	62.31%
Pos	23.56%	15.09%	22.39%	34.86%
UOR	2.49%	2.07%	2.50%	2.83%

UOR: unable to obtain results



Conclusions

The Epi proColon® test is a robust assay for molecular screening in CRC cancer patients using plasma specimen type.

References

1. Epi proColon® Instructions for Use. IFU 008, rev 10, April 2016.