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

**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, as defined by 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.

**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 26.06% 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 plasma. 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 16.23% with females at 13.35% 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.

**Repeatability**

Repeatability (intra-assay precision) was 100% 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 pools were 100% concordant
- 3 clinically negative pools were 100% concordant
- 24/25 (96%) replicates had 1 Septin9 PCR positive sample in 3 replicates and sensitivity was 96%
- Overall concordance 0.96

**Reproducibility**

Reproducibility (inter-assay precision) was 100% 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 pools were 100% concordant
- 3 clinically negative pools were 100% concordant
- 24/25 (96%) replicates had 1 Septin9 PCR positive sample in 3 replicates and sensitivity was 96%
- Overall concordance 0.96

**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**

**Conclusions**

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

**References**