

mSEPT9 Clinical Feasibility Data

SEPT9 methylation in CRC tissue. The SEPT9 methylation assay is based on CpG sites that are aberrantly methylated in the promoter region of the v2a transcript of the SEPT9 gene as determined in CRC tissue studies. Figure 4 shows that over 90% of CRC tissues are methylated in this gene region.

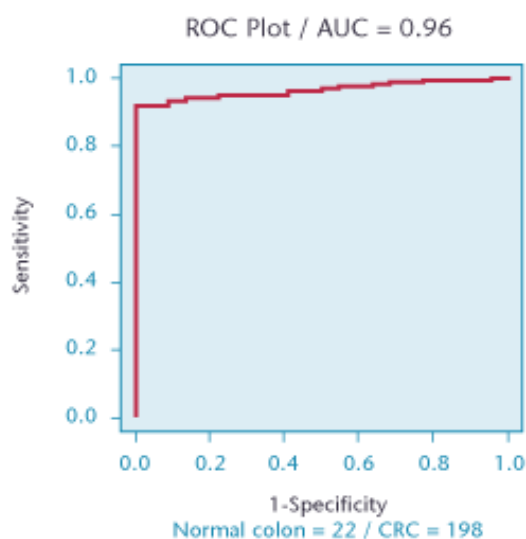


Figure 4. Performance of SEPT9 in colorectal cancer and healthy colon tissue.

*m*SEPT9 research assay. Epigenomics' initial research workflow for plasma DNA analysis was based on Roche instrumentation and was used for all plasma feasibility studies prior to 2008. The DNA extraction step was performed using the Roche MagNaPure automated extraction device, followed by a manual bisulfite treatment step and real-time PCR using the Roche LC 1.2 or 2.0.

In the *m*SEPT9 assay, the presence of the methylated v2 region is determined through 3 separate amplifications of the final DNA material extracted and bisulfite treated from a 4-6 ml plasma sample. A control assay is used to qualify each sample by requiring a minimum concentration of total bisulfite DNA. The *m*Sept9 assay detects the presence of methylated SEPT9 DNA in the patient plasma sample and the data is reported as a binary variable (methylated SEPT9 DNA present vs. absent). In the results of early investigations, the interpretation of the result was based on the analysis of the resulting 3 PCR amplification curves according to several decision rules. High specificity performance could be achieved if two or more of the 3 separate PCR reactions were required for a positive result. High sensitivity performance could be achieved if only one of the three reactions were required for a positive result. A conditional algorithm has recently been developed that takes into consideration the amount of total DNA in the PCR reaction as compared to the number of positive measurements. This algorithm results in both improved sensitivity and specificity of the test.

Clinical Proof-of-Concept. Feasibility studies for the test were performed on plasma

samples from individuals undergoing colonoscopy for various reasons. The individual may or may not have had symptoms of disease. Blood was typically drawn at least a week after colonoscopy and sometimes prior to colonoscopy.

Figure 5 shows results of a study in which three markers, including SEPT9, were tested for presence of methylation in plasma. The data are displayed as box-percentile plots of DNA concentrations in plasma for colonoscopy-verified normal patients (normal) and patients with CRC. Concentrations of methylated DNA are shown for the TMEFF2, NGFR and SEPT9 markers. Concentration of total bisulfite DNA (bisDNA) is also shown for all samples. Median DNA concentrations are red horizontal lines; 25th and 75th percentiles are blue horizontal lines. The width of the box-percentile plot at any given height is proportional to the percent of observations that are more extreme in the direction leading away from the median. Individual measurement values are plotted as grey circles. In this study the difference between the median DNA concentrations for normal and CRC are the greatest for SEPT9 and the distribution of methylated DNA concentrations for *m*SEPT9 can be seen to be higher than for the other markers. This initial study provided sufficient evidence that SEPT9 methylated DNA could be found in patients with CRC and in great enough levels to warrant further study of this marker as a potential CRC screening tool.

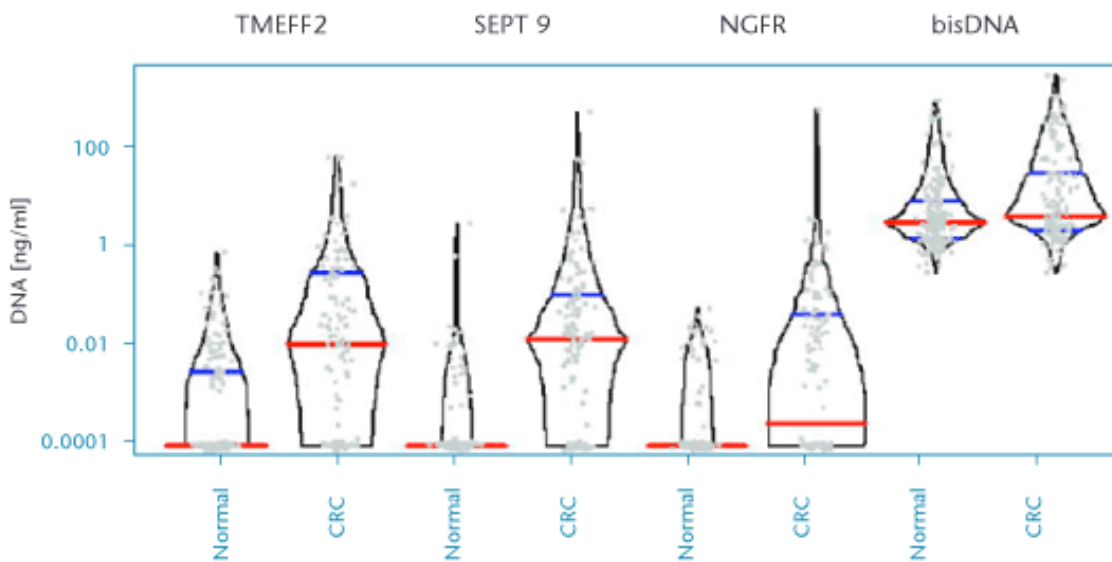


Figure 5. Presence of methylated DNA in plasma as measured by TMEFF2, SEPT9 and NGFR. Bisulfite DNA levels are also indicated.

SEPT9 validation. Further verification of selected markers is typically conducted by performing a training case control study to determine the best classifier for the assay performance followed by testing of the classifier on a masked, independent case control sample set. Any major changes in the assay require additional testing in this manner. Through this process we have tested over 2600 plasmas from CRC patients, healthy individuals and other disease controls using the *m*SEPT9 assay.

The quality of the sample processing in both studies was very high and demonstrated consistent recovery of both plasma DNA and bisulfite converted DNA.

The *m*SEPT9 biomarker shows consistent results in three independent studies with very similar sensitivity results for each cancer stage and similar specificity in the control population even though through workflow improvements Epigenomics has reduced sample volume and implemented entirely different extraction, bisulfite treatment

procedures, and PCR probe type. (Figure 6).

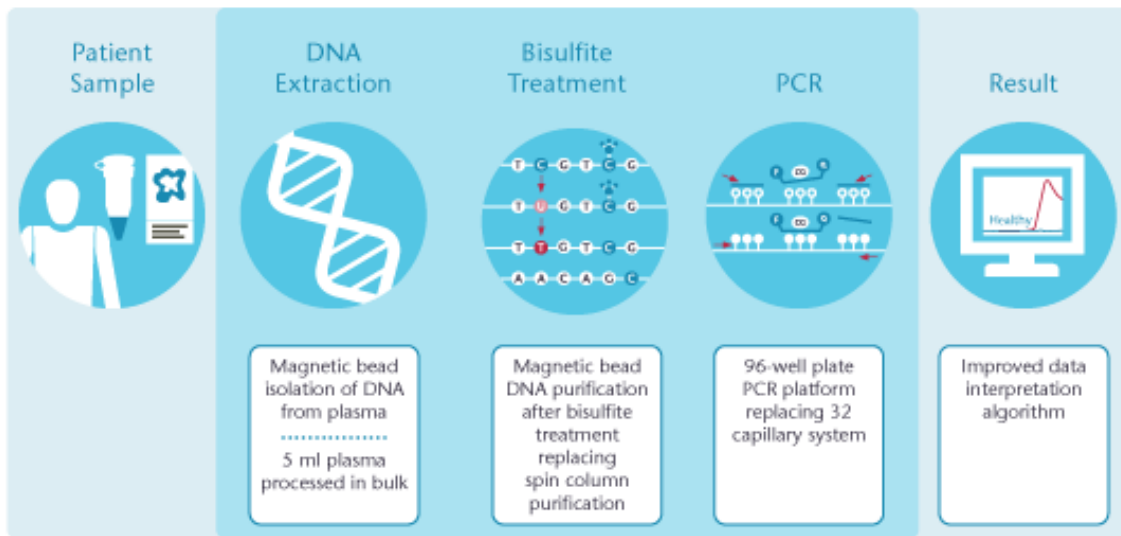


Figure 6. Improved "routine-friendly" assay for Septin 9 methylation detection in blood plasma

Based on these data, the *m*SEPT9 test appears to offer significant advantages over currently recommended methods for CRC screening use: it is minimally invasive, has effective early stage disease detection, and has good specificity. If confirmed in an average (to increased) risk population eligible for CRC screening, these characteristics would appear to support its use as a first-line screening test.