

Technical Validation and Field Study of a Research Use Only (RUO) Kit for Detection of Methylation of the ^mSEPT9 DNA Biomarker in Plasma

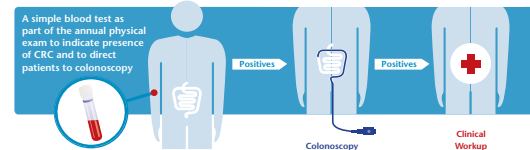
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Introduction

Colon cancer is the second-leading cause of cancer death in adults in the United States (1) despite a survival rate of over 90% when detected early (2). Chances for cure drop rapidly once colon tumors have spread beyond the confines of the bowel.

Currently, less than half of guideline eligible individuals are willing to undergo regular screening for CRC, typically a colonoscopy or stool based test, highlighting the need for a new test that will drive patient compliance. This could be achieved by a blood test for colorectal cancer (3).

We previously identified and verified methylated SEPT9 DNA (^mSEPT9) as a blood-based biomarker for all stages of CRC with favorable clinical performance characteristics in several clinical sample studies (4). Two prospective case control studies with 518 samples were performed (Table 1). In the first study (269 subjects) 72 of 97 cancer cases (74% sensitivity) were detected with 92% specificity. The second case control study (249 subjects) confirmed the performance observed in the first study. In this study 63 of 91 colorectal cancer patients of all stages were identified (69% sensitivity) with 89% specificity. Both studies used a research grade workflow. The availability of a standardized kit could greatly facilitate ^mSEPT9 in the laboratory routine. Such a kit would have to cover all required process steps from plasma DNA extraction and subsequent bisulfite treatment to real time PCR analysis with suitable workflow controls.



	^m SEPT9 Detection Assay Training Study 2008 (N = 269)		^m SEPT9 Detection Assay Testing Study 2008 (N = 249)	
Stage I	10/22	45	10/20	50
Stage II	31/37	84	29/40	72
Stage III	28/35	80	20/27	74
Stage IV	3/3	100	4/4	100
Stage I-III	69/94	73	59/87	68
Stage I-IV	72/97	74	63/91	69
Controls	13/172	8	17/158	11

Table 1. ^mSEPT9 Performance in Blood Samples: Case-Control Studies

Methods

To facilitate routine laboratory use and to allow for comparison of performance at different laboratories, a standardized kit including external calibrators and workflow controls was developed for the ^mSEPT9 biomarker (Figure 1).

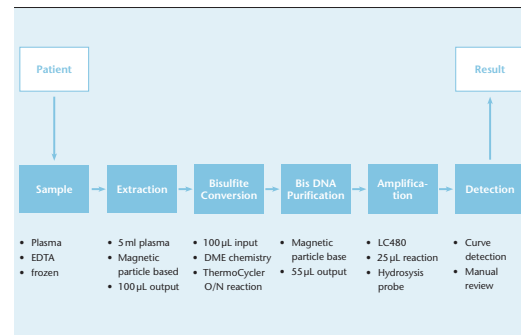


Figure 1. Schematic Workflow of the ^mSEPT9 kit.

The kit comprises components to perform plasma DNA extraction, bisulfite conversion of DNA, purification of bisulfite-converted DNA, quantification of converted DNA by real-time PCR, and measurement of ^mSEPT9 methylation by real-time PCR using the LightCycler[®] 480.

The analytical performance of this research kit was characterized by analyzing technical samples with very low concentrations of ^mSEPT9. Plasma samples spiked with methylated DNA of known concentrations were used. 40 replicates were analyzed for each concentration of spiked methylated DNA: 0 pg/ml spike; 30 pg/ml spike; 120 pg/ml spike; 360 pg/ml spike and 1080 pg/ml spike). Calibration was performed using an external standard curve. Workflow controls for the entire process as well as PCR controls were established and validated for routine use. Robustness and variability of the assay was determined using different operators, kit lots, and lab conditions. As a final confirmation a field study was performed on 97 plasma samples obtained from clinical gastroenterological sites. This study processed the clinical specimens using the ^mSEPT9 research workflow and in parallel the ^mSEPT9 Detection Assay Kit for methylation analysis.

Disclaimer: The ^mSEPT9 Research Use Only (RUO) Kit is available for purchase and sale in the European Union only. The U.S. Food and Drug Administration has neither established the safety and effectiveness, nor evaluated the performance characteristics, of the ^mSEPT9 RUO Kit.

Results

A new commercially available ^mSEPT9 Detection Assay Kit has been developed. This assay was characterized and the performance was compared with the research grade assay used in previous case control studies. Based on the analysis of diluted spiked plasma, a theoretical LoD of 30 pg/ml methylated DNA (approx. 9 copies) in plasma was identified (Figure 2). This result was verified by detecting 30 pg/ml in 37 of the 40 determinations (92.5%). The LoB was determined to be 0 pg/ml (0 of 40 replicates positive) plasma. The variability of the assay was found to be remarkably low with a coefficient of variance (CV) of the determined crossing point (CP) of CV=0.24 at the lowest concentration. The variability of both assays was substantially equivalent (Figure 3). An independent field study was performed to evaluate the kit for stability and ease of use under routine clinical lab conditions. In this study the assay was found to be stable with 98.9% (96/97) valid measurements. Results of the field study demonstrated a percentage of overall agreement of the results of both assays of 81% (Figure 4).

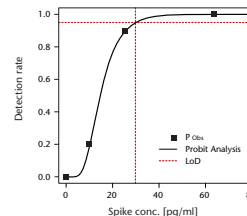


Figure 2. Estimation of the limit of detection. X-axis: concentration methylated DNA spike in bulk plasma [pg/ml]; Y-axis: probability for a positive test result using the ^mSEPT9 assay.

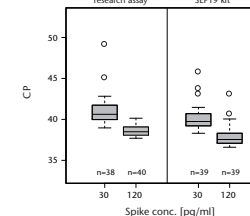


Figure 3. Analysis of the variability of the new ^mSEPT9 kit and the ^mSEPT9 research assay. X-axis: concentration methylated DNA spike in bulk plasma [pg/ml]. The numbers indicate the call rate of 40 determinations; Y-axis: box-plot of the determined crossing points (CP) of both assays.

		^m SEPT9 kit	
^m SEPT9 research assay	-	7	40
	+	38	11
		+	-

Figure 4. Side-by-side comparison of the new ^mSEPT9 kit and the ^mSEPT9 research assay (CRC samples: 50; Controls: 47 self-reported).

Conclusions

The ^mSEPT9 Detection Assay Kit provides a reliable tool to assess the presence of methylated DNA of the SEPT9 gene in standard plasma samples obtainable in routine clinical practice. The information generated with the assay should be valuable for furthering research of colorectal cancer detection in plasma and help to establish a strategy for early detection of colorectal cancer in blood samples. Its ease of use and utilization of instrumentation available in most research and clinical laboratories along with reproducible performance results should help standardize clinical research into blood based colorectal cancer detection.

Prospective Study: PRESEPT

The performance characteristics of the biomarker ^mSEPT9 are currently being investigated in the PRESEPT Study. PRESEPT is a prospective, multi-center study performed in a U.S. CRC screening guideline-eligible population. The study is expected to enroll 7,500 average and increased risk screening guideline-eligible individuals already scheduled for colonoscopy, a population expected to yield about 50 cancer cases. The PRESEPT data will be used to analyze the performance and the potential health economic benefit of ^mSEPT9 blood testing in population-wide CRC screening.

The study is expected to be finalized with first data being published by the end of 2009.

For more information visit www.presept.com.

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