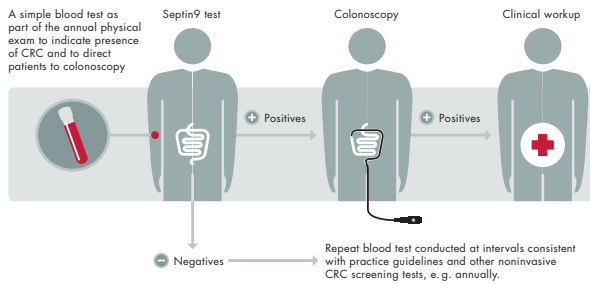


A New Duplex Real-time PCR Assay for Detection of the ^mSEPT9 Biomarker for Colorectal Cancer Screening Using Blood Plasma

Gunter Weiss, Philipp Schatz, Anne Fassbender, Ina Fuhrmann, Reimo Tetzner
Epigenomics AG, Kleine Präsidentenstr. 1, 10178 Berlin, Germany

Background

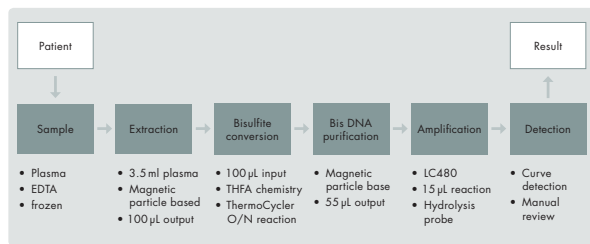
Despite the good prognosis for colorectal cancer patients when disease is detected early, compliance to screening programs remains low. Concomitants and inconvenience of available methods point to the need of convenient and reliable tests with the potential to increase patient adherence. Previously we have shown in more than 3,000 patient plasma samples that the detection of methylated DNA of the SEPT9 gene (^mSEPT9) is strongly associated with the presence of colorectal cancer. Here, we present data generated with a improved workflow that utilizes a duplex real-time PCR that greatly simplifies reliable ^mSEPT9 detection in human plasma.



Materials and Methods

DNA from human plasma is extracted, bisulfite converted, and finally purified with the workflow developed by Epigenomics. The output DNA is suited for detection via real-time PCR. Detection of DNA is accomplished via a duplex PCR combining a highly sensitive methylation specific SEPT9 DNA detection assay with a beta-actin assay used as an internal control. The entire workflow has been optimized to increase robustness and improve the ease-of-use. Comparative data were generated on technical samples prepared from human plasma and spiked with concentrations of methylated DNA (mDNA). Plasma aliquots with a dilution series of mDNA spikes were measured repeatedly. In addition, 80 sample aliquots derived from clinical patient material were measured. The results were compared to data generated by the published reference method (^mSEPT9 Detection Assay, Epigenomics). The performance of ^mSEPT9 for the average to increased risk population is currently being evaluated in the multi-center clinical study PRESEPT.

Workflow of the Duplex Real-time PCR Assay



Results

Both workflows consistently detected a ^mSEPT9 signal ($\geq 95\%$ of replicates) in aliquots with a 8 pg/ml spike of mDNA. The observed standard deviation for repeated measurements was slightly lower for the new duplex workflow when compared to the reference method. For the clinical samples we observed excellent agreement of results between the two methods in 85% of the valid cases (60/80).

^mSEPT9 validated in over 3000 plasma samples

Study	# Samples	Sensitivity	Specificity	Workflow
Study 1 ¹⁾	312	52	95	Experimental workflow
Study 2 ¹⁾	600	57	96	
Study 3 ¹⁾	725	48	93	
Study 4 ²⁾	370	48	96	
Study 5 ²⁾	550	72	90	
Study 6 ³⁾	269	73	93	^m SEPT9 Detection Assay
Study 7 ³⁾	245	69	89	
Total	3071			

Figure 1. Overview of Case Control Studies in Plasma Using ^mSEPT9

1) DNA methylation biomarkers for blood-based colorectal cancer screening (2008), Lofton-Day, C., et al. *Clinical Chemistry* 54, 414-423
2) Sensitive Detection of Colorectal Cancer in Peripheral Blood by Septin 9 DNA Methylation Assay (2008), Gratzmann, R. et al., *PLoS ONE*, Volume 3, Issue 11, e3759
3) Circulating Methylated SEPT9 DNA in Plasma is a Biomarker for Colorectal Cancer; DeVos, T. et al. (2009), *Clinical Chemistry*, 55:7, 1337-1346 (2009)

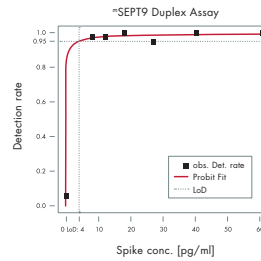


Figure 2. Estimation of the limit of detection. X-axis: concentration of methylated DNA spike in bulk plasma [pg/ml]; Y-axis: probability for a positive test result using the ^mSEPT9 Duplex Assay.

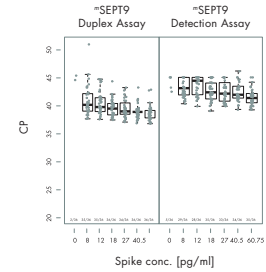


Figure 3. Analysis of the variability of the new ^mSEPT9 Duplex Assay (left panel) and the ^mSEPT9 Detection Assay (right panel). X-axis: concentration of methylated DNA spike in bulk plasma [pg/ml]; The numbers indicate the call rate within 36 PCR determinations per concentration; Y-axis: box-plot of the determined crossing-points (CT) for ^mSEPT9 PCR.

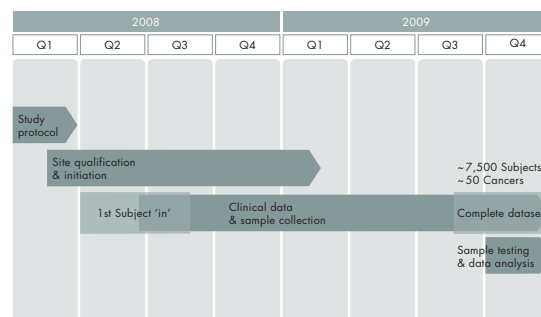
Conclusions

The biomarker ^mSEPT9 has been established in several independent studies to be ~70% sensitive to detect colorectal cancer at ~90% specificity. The newly developed duplex workflow for ^mSEPT9 detection in human plasma shows excellent agreement with, and therefore is considered substantially equivalent to, the reference method. This improved assay, which has been demonstrated to accurately detect ^mSEPT9 in a standard blood specimen, is expected to significantly improve patient management. The design and robustness of the assay will enable its use in standard routine laboratory procedures.

Goals of the Prospective Study PRESEPT

- Establish ^mSEPT9 performance characteristics for average to increased risk population in an international, multi-center, prospective study (USA and Germany)
- Collect samples and data in compliance to ICH GCP and capable of supporting product regulatory applications
- Demonstrate health economic benefit to support national healthcare and private insurer coverage
- Obtain medical community acceptance of ^mSEPT9 assay for CRC screening and gain inclusion into multi-societal screening guidelines

Timeline – PRESEPT Study



- 25 clinical sites in US and Germany actively enrolled
- > 6,000 subjects enrolled (September 2009)
- Visit www.presept.net for regular updates on study progress.

*PRESEPT Clinical Study Steering Committee

Advises on study design, oversees study conduct, and will independently analyze and accurately report final results. The CSSC membership composition:

- David Ransohoff, MD, Professor of Medicine, Cancer Epidemiology, Cancer Prevention and Control, University of North Carolina School of Medicine, CSSC Chair
- Neal Osborn, MD, Co-Director of Clinical Research, Atlanta Gastroenterology
- Timothy Church, PhD, Professor, School of Public Health, University of Minnesota
- Brent Blumenstein, PhD, Principal, Trial Architecture Consulting
- Dale Snover, MD, Adjunct Professor, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School
- Prof. Thomas Rösch, M.D, Director of the Department of Interdisciplinary Endoscopy University Hospital Hamburg-Eppendorf, Germany
- Robert Day, MD, PhD, President Emeritus of The Fred Hutchinson Cancer Research Center (ex officio member)
- Michael Wandell, PharmD, Study Director, Epigenomics
- Cathy Lofton-Day, PhD, Project Manager, Epigenomics