

# Clinical Studies Verify Performance of the Blood-based Septin 9 DNA Methylation Assay for the Detection of Colorectal Cancer



AUTHORS: Cathy Lofton-Day<sup>1</sup>, Theo deVos<sup>1</sup>, Matthias Schuster<sup>2</sup>, Andrew Sledziewski<sup>1</sup>, Michael Wandell<sup>1</sup>, Thomas Rösch<sup>3</sup>

<sup>1</sup> Epigenomics, Inc. 1000 Seneca St, Ste 300 Seattle, WA 98101, USA; <sup>2</sup> Epigenomics AG, Kleine Präsidenten Str. 1, 10178 Berlin, Germany;

<sup>3</sup> Universitätsklinikum Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

## Introduction

Colorectal cancer (CRC) is the second leading cause of cancer deaths despite a survival rate of over 90% when detected early. Currently, less than half of guideline eligible individuals are willing to undergo regular screening for CRC, typically using colonoscopy or stool based tests, highlighting the need for a new test that will drive patient compliance. We previously identified and verified methylated Septin 9 DNA as a blood-based biomarker for all stages of CRC. Here we show data of the latest multi-center case control study and progress of the ongoing prospective clinical validation study PRESEPT.

## Methods and Results

A new clinic-ready assay for Septin 9 DNA methylation in blood plasma was used to further verify the biomarker for colorectal cancer detection. Two prospective case control studies with 518 samples were performed. The first study (269 subjects) tested blood samples from 97 patients with CRC, and 172 individuals without colorectal cancer as confirmed by colonoscopy. The new assay detected 72 of 97 cancer cases (74% sensitivity) with 92% specificity. The second case control study, composed of an entirely independent set of blood plasma samples from 249 subjects, confirmed the performance observed in the first study. In this study the clinic-optimized Septin 9 assay identified 63 of 91 colorectal cancer patients of all stages (69% sensitivity) with 89% specificity. In these two independent studies, the performance of the optimized assay was statistically equivalent to the performance of the research assay previously used in a 2006 study of over 300 subjects, which demonstrated a sensitivity of 72% at a specificity of 90%. Further we are verifying Septin 9 in a true screening population in prospective PRESEPT study.

PRESEPT is a multi-center clinical study sponsored by Epigenomics to prospectively evaluate the clinical performance of methylated Septin 9. It is one of the first studies to evaluate the performance of a non-invasive test to indicate presence of colorectal cancer in colorectal cancer screening guideline-eligible individuals in a standard blood sample. The study is designed to enroll up to 7,500 subjects aged 50 or older at average to increased risk for colorectal cancer who have been scheduled for a screening colonoscopy at multiple clinical sites in the U.S. and Germany. This population is expected to harbor about 50 cases of undetected colorectal cancer. Enrollment for the study is expected to be finished in 2009 and Septin 9 testing of the prospectively collected samples by an independent clinical laboratory will follow.

## Conclusions

With more than 3000 patient plasma samples tested, methylated Septin 9 DNA is one of the most extensively studied blood-plasma marker for the detection of colorectal cancer. A commercially available blood based test for colorectal cancer has the potential to change the CRC screening landscape since it eliminates patient involvement in sample collection, and gives the control into physicians routine.

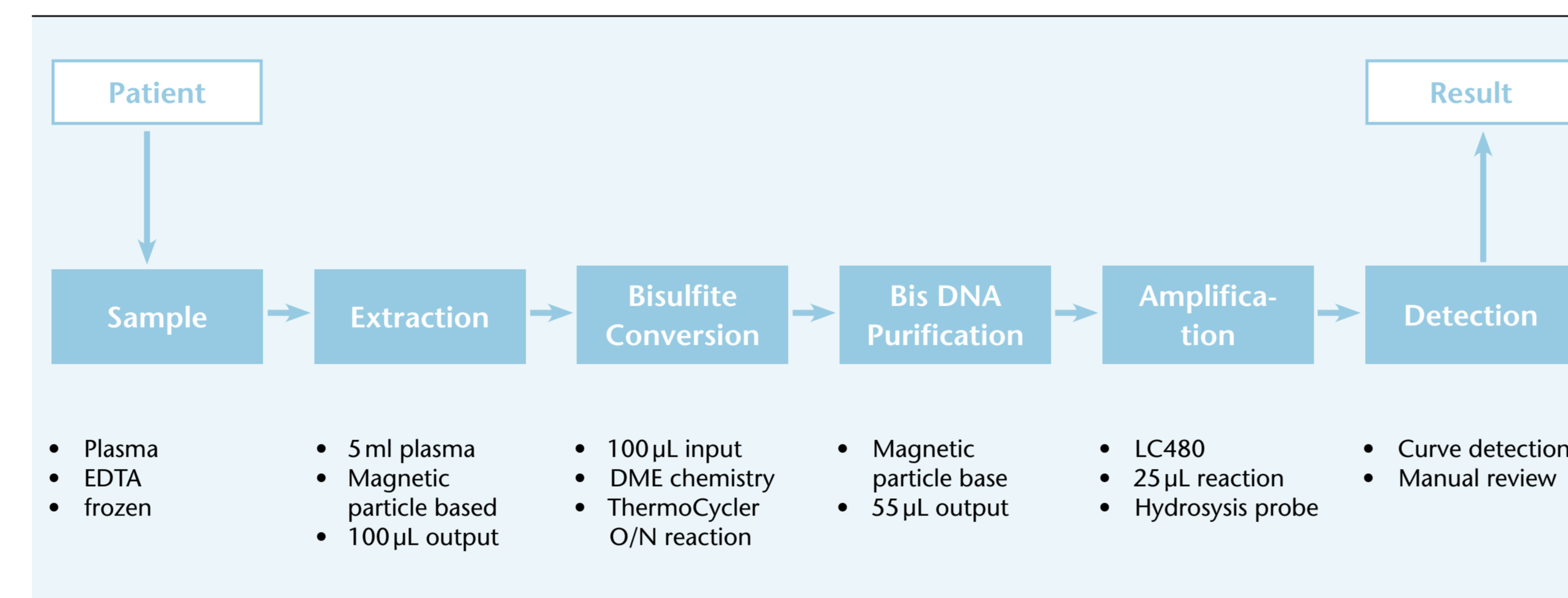


Figure 1. Outline of the <sup>m</sup>SEPT9 assay workflow. The assay was optimized for an input volume of 4–5 mL of plasma. Extraction of DNA from plasma and purification of DNA following bisulfite treatment use magnetic particle methods. Assay results can be obtained within 32 hours from the start of sample processing.

## <sup>m</sup>SEPT9 validated in over 3000 plasma samples

Study	# Samples	Sensitivity	Specificity	Workflow
Study 1 <sup>1)</sup>	312	52	95	Experimental Workflow
Study 2 <sup>2)</sup>	600	57	96	
Study 3 <sup>3)</sup>	725	48	93	
Study 4 <sup>2)</sup>	370	48	96	
Study 5 <sup>2)</sup>	550	72	90	<sup>m</sup> SEPT9 Detection Assay
Study 6 <sup>3)</sup>	269	74	92	
Study 7 <sup>3)</sup>	249	69	89	
Total	3071			

Table 1. Overview of Case Control Studies in Plasma Using SEPT9

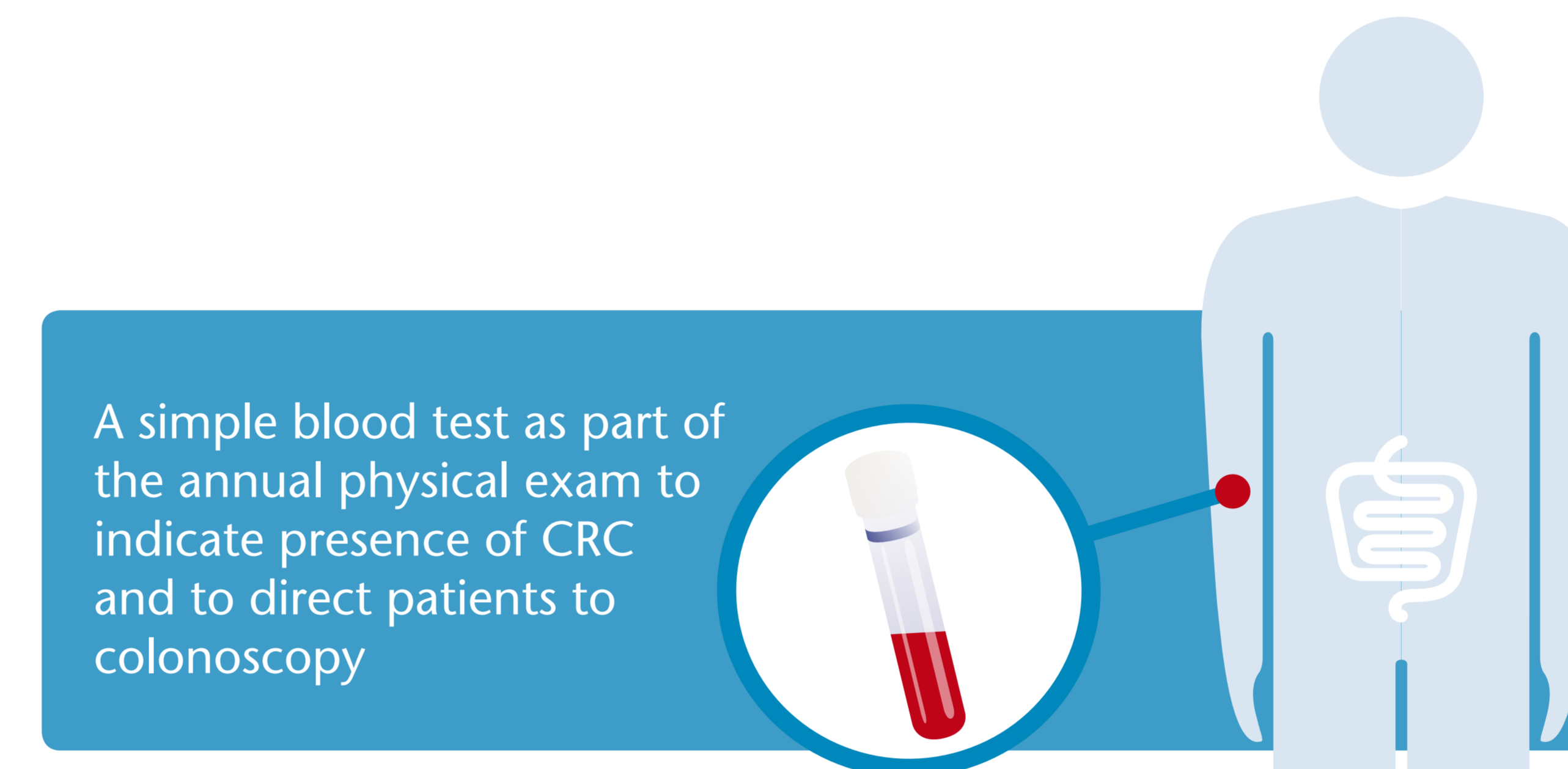
<sup>1)</sup> DNA methylation biomarkers for blood-based colorectal cancer screening (2008). Lofton-Day, C., et al. *Clinical Chemistry* 54, 414–423

<sup>2)</sup> Sensitive Detection of Colorectal Cancer in Peripheral Blood by Septin 9 DNA Methylation Assay (2008). Gruetzmann, R. et al., *PLoS ONE*, Volume 3, Issue 11, e3759

<sup>3)</sup> Circulating Methylated SEPT9 DNA in Plasma is a Biomarker for Colorectal Cancer; DeVos, T. et al. (2009), *Clinical Chemistry*, online and in print

Patient Group	<sup>m</sup> SEPT9 Detection Assay Training Study 2008 (N = 269) <sup>1)</sup>		<sup>m</sup> SEPT9 Detection Assay Testing Study 2008 (N = 249) <sup>1)</sup>	
	Positive/Tested	% Positive	Positive/Tested	% Positive
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 2: <sup>m</sup>SEPT9 Performance in Blood Samples: Case-Control Studies



A simple blood test as part of the annual physical exam to indicate presence of CRC and to direct patients to colonoscopy

## Goals of the PRESEPT Initiative

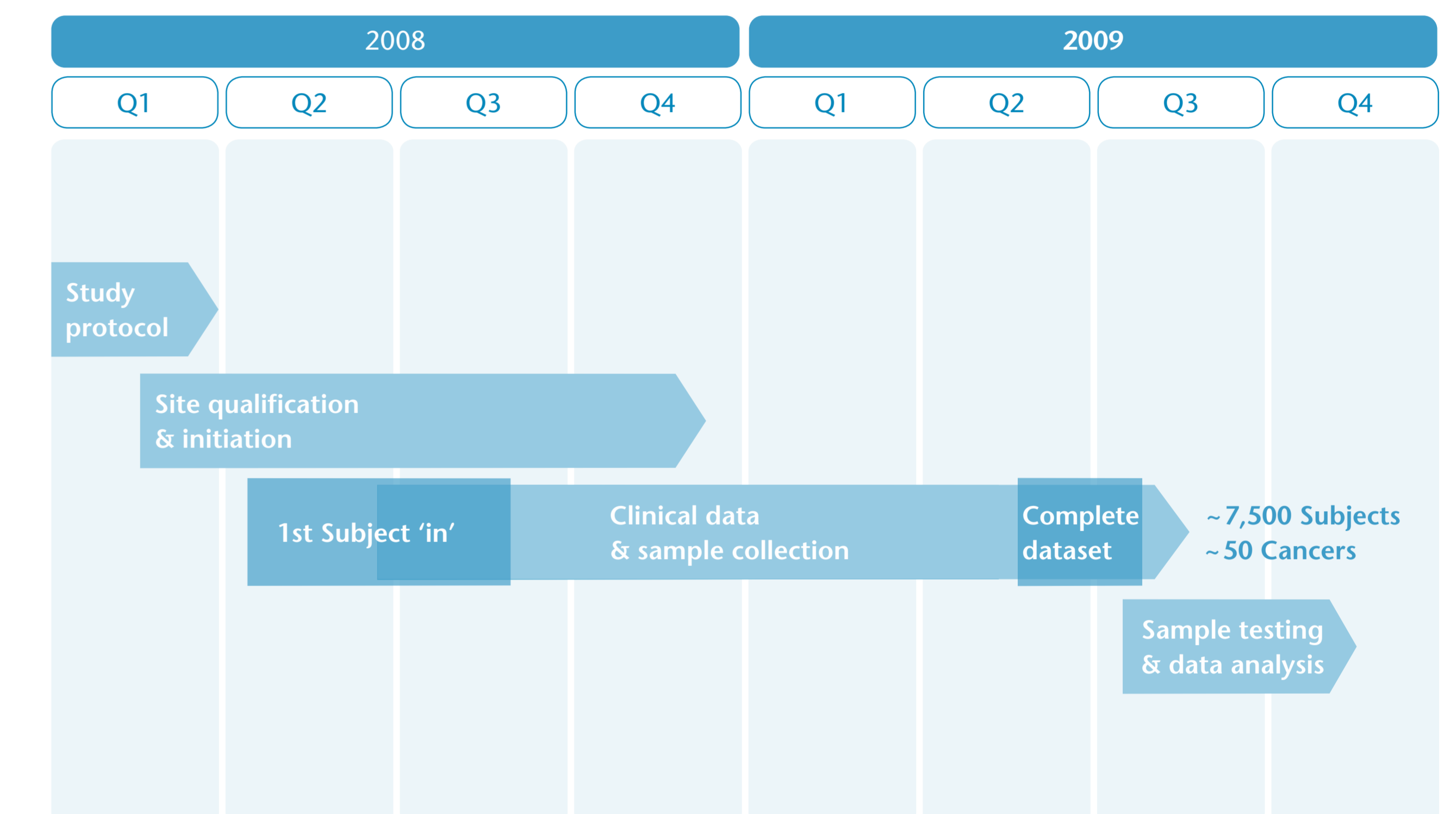
- Establish <sup>m</sup>SEPT9 performance characteristics for average to increased risk population in an international, multi-center, prospective study (USA and Germany)
- Collect samples and data according to ICH GCP supporting IVD PM approval
- Demonstrate health economic benefit to support national healthcare and private insurer coverage
- Obtain medical community acceptance of <sup>m</sup>SEPT9 assay for CRC screening and gain inclusion into multi-societal screening guidelines

## Prospective Study: PRESEPT

<sup>m</sup>SEPT9 performance characteristics in an average to increased risk population

- CRC screening guideline-eligible subjects
  - Asymptomatic individuals at average to increased risk for CRC
  - 50 years of age or older
- Age and gender accrual targets to reflect target population
- **Primary Endpoint:** Clinical/surgical diagnosis of invasive colorectal adenocarcinoma detected by optical colonoscopy and confirmed by histology compared to the <sup>m</sup>SEPT9 biomarker classification.
- **Secondary Endpoint:** Detection of polyp(s) equal to or greater than 10 mm, flat lesion(s), or non-invasive adenocarcinoma (CIS) by colonoscopy and confirmed by histology compared to the <sup>m</sup>SEPT9 biomarker classification will also be described.

## Timeline – PRESEPT Study



Visit [www.presept.net](http://www.presept.net) for regular updates on study progress

- Protocol approved by Medical Advisory Board (Douglas Rex, MD, Philip Schoenfeld, MD, Deborah Fisher MD, Scott Ramsey, MD, Richard Wender, MD)
- IRB approvals obtained
- Established Clinical Study Steering Committee\*
- 22 clinical sites in US and Germany actively enrolling
- > 4000 subjects enrolled (May 09)

## \*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