



PRESEPT Study Design & Details

PRESEPT objectives. The overarching objective of the PRESEPT initiative is to establish *m*SEPT9 as a non-invasive CRC screening method and to make this test available for routine testing as a simple blood test, performed e.g. as part of a regular medical examination. We will prospectively collect patient plasma samples and clinical information to support development of clinical laboratory test services for routine medical testing by Epigenomics' partners. It is currently envisioned that *m*SEPT9 routine testing is first expected to be offered as a Laboratory Developed Test (LDT) and later as an IVD by Abbott Molecular and further partners of Epigenomics. Plasma and clinical data collection activities will furthermore be aligned to obtain FDA agreement to a clinical plan supporting the IVD according to the anticipated premarket approval application (PMA) process of our partner(s) and will therefore be conducted under Good Clinical Practice (GCP). A further goal of the PRESEPT initiative is to integrate the data of the PRESEPT Study into a model analyzing the health economic impact of *m*SEPT9 testing. All key aspects of the PRESEPT initiative, i.e. lab test service and IVD development, are directed to ultimately achieve acceptance of the test for CRC screening by the medical community and patient advocacy groups. Accomplishment of widespread adoption will be evidenced by inclusion of the test into CRC screening guidelines of medical societies and coverage of the test by health care providers.

The main goal of the PRESEPT initiative is to demonstrate the utility of the *m*SEPT9 assay for CRC screening. The PRESEPT Study will address this goal by evaluating the performance of the *m*SEPT9 assay for the detection of CRC in individuals at an average to increased risk for CRC in a prospective multi-center trial. Epigenomics intends to enroll approximately 7,500 individuals aged 50 and older in the United States and Germany in order to collect a population with an age, gender and ethnic profile reflective of the U.S./German CRC screening population.

PRESEPT Study population. The primary objective of the investigation is to evaluate and validate the clinical performance of the *m*SEPT9 assay for the detection of colorectal adenocarcinoma (Stage I-IV) in CRC screening guideline eligible individuals. Individuals, in this study, are screening guideline eligible if they are 50 years of age or older, and at average to increased risk for CRC. Selected for this investigation should be representative of the US/German screening guideline eligible population and should have been scheduled for screening and not for diagnostic colonoscopy. As a second objective, the performance of the *m*SEPT9 assay for the detection of adenomatous polyps ≥ 1 cm will be assessed.

We note that our proposed study population will, in addition to individuals at average, age-related risk for CRC, encompass individuals with an increased risk for CRC. This group of increased risk individuals is made up of a subset of individuals with a family history of CRC. A familial risk, i. e. two or more first- or second degree relatives, is thought to be present in up

to 20% of all patients with CRC.²⁴ In our study protocol, we explicitly evaluate familial risk and exclude individuals with 2 or more 1° relatives with CRC or with 1 or more 1° relative(s) <50 years as well as individuals with the established diagnosis of Hereditary Nonpolyposis Colorectal Cancer (HNPCC) or Familial Adenomatous Polyposis (FAP). We do not exclude individuals with a weaker familial risk such as individuals with only one 1° relatives with CRC (50 years or older) or with any 2° or 3° degree relative(s). Thus, we only exclude individuals whose CRC is likely or proven (for HNPCC, FAP) to be inherited as a Mendelian trait.

Subject enrollment process. The PRESEPT approach to identify representative, CRC screening guideline eligible individuals, who qualify to be included into the analysis follows a two step procedure:

In a first step, screen individuals are evaluated for general eligibility for PRESEPT.

Briefly, an individual is eligible if he/she is at least 50 years of age, scheduled for screening colonoscopy (as opposed to diagnostic purposes), shows a general interest in the study and is capable of providing informed consent. Individuals meeting all four of these criteria will be enrolled.

Subsequently, in a second step, it is determined if individuals, rendered eligible in the first step, meet pre-defined inclusion and exclusion criteria. The decision to finally include individuals, already enrolled in the first step, into the analysis will be dependent that any of the inclusion and none of the exclusion criteria are fulfilled. Note that 'Eligibility' and 'Inclusion/exclusion' are not used synonymously in this study.

Inclusion criteria are:

- Informed consent form given
- Guideline eligibility for screening colonoscopy
- Blood draw prior to initiation of bowel preparation
- Scheduled colonoscopy is first large bowel endoscopy in lifetime

Exclusion criteria are:

- Reported family history of CRC (defined as 2 or more 1° relatives with CRC; 1 or more 1° relative(s) < 50 years with CRC; known HNPCC or FAP)
- Rectal bleeding within the last six months for which the individual has sought medical attention
- Anemia within the last six months for which the individual has sought or received medical care
- Previous history of colorectal polyps or CRC.

Following the advice of one of Epigenomics' Medical Advisory Board members ([detailed in Ref 25](#)), exclusion criteria were defined more stringently with respect to disease symptoms and signs than in other published studies. 'Changes in bowel habits', 'anorectal bleeding anytime' and 'abdominal pain' are therefore not listed as exclusion criteria as they are rather unspecific and represent complaints common in the age groups of the population addressed in this study. Excluding individuals with these unspecific symptoms would lead to elimination of many (enrolled) subjects

that have passed the first step and finally result in a study population not representative of the screening guideline eligible population.

For the successful demonstration of the utility of the Septin 9 assay for CRC screening, it is critical to have a clear understanding of screening colonoscopy eligibility and to assure that the study is conducted with individuals scheduled for screening and not for diagnostic colonoscopies. Determination of screening colonoscopy in this investigation is entirely based on expert opinion and judgment and not on the Sponsor's conclusions of clinical data obtained from study participants. This is particularly important for the (screening) step evaluating eligibility as this is the first filter for the identification of participants.

'Screening colonoscopy' will initially be evaluated as part of the set of questions asked during the screening step by the responsible personnel, either by personal interview or by phone, following an approved script. A patient is also considered in the 'screening colonoscopy' group if the referring physician has classified the procedure as screening. What ultimately defines screening colonoscopy in the individual undergoing the procedure is that the colonoscopy was motivated by CRC screening and not by the presence of symptoms. Should the history reveal abdominal symptoms (not listed as exclusion criteria) this individual would still qualify as 'screening colonoscopy patient' since she/he has initially sought medical advice for screening purposes. In other words, abdominal symptoms in this individual would be revealed ex post after scheduling for screening colonoscopy had occurred.

Expected PRESEPT Study population. Using this approach, a CRC prevalence of 0.6 – 0.7% in the study population is expected. Subjects will be accrued until at least 50 CRC cases are identified. For the detection of 50 subjects with CRC, it is anticipated to enroll and include approximately 7,500 individuals in the United States and Germany. Age stratification is applied to avoid under enrollment of subjects 70 years and older who are underrepresented in the colonoscopy screening population as compared to the US census. It will be one of the responsibilities of the Sponsor to coordinate study activities to assure that these accrual targets are met across all participating clinical sites.

Blood plasma sampling process. Following enrollment and evaluation of inclusion/exclusion criteria by health history interview, 40 ml of blood will be drawn from each subject. Depending on the specific workflow at the individual clinical site, blood may be drawn at the same location of the preceding steps, i. e. informed consent, health history, or at a remote location. Similarly, blood draw may be drawn by study personnel or may involve in-house blood draw-stations. Epigenomics has anticipated differences in the clinical routine workflows between clinical sites and therefore designed all study-related documentation accordingly. The requested clinical data has a modular set-up and is split into five different Case Report Forms (CRFs) reflecting that data capture and specimen collection may be performed by different personnel at different locations. Regardless of potential de-centralized study activities, it will be the responsibility of the Principal Investigator or designee to co-ordinate these activities and centralize data and specimens as outlined in the protocol. Another form, the Subject Management Form, was designed to facilitate

the coordination of all activities from enrollment to completion and to record the relevant steps and subject status.

Septin 9 testing. Once collected, the blood plasma samples will be frozen until they are processed for SEPT9 DNA methylation testing by Epigenomics or one or more of its partners developing IVD tests for the *m*SEPT9 biomarker. The samples are expected to be processed in one or few batches after the full number of samples to obtain 50 cancer cases has been collected. The Clinical Steering Committee will decide on the samples to be included in the *m*SEPT9 analysis.