2018-10 Protocol V.2

An Evaluation of a Multi-Target Stool DNA (mt-sDNA) Test, Cologuard, for CRC Screening in Individuals Aged 45-49 and at Average Risk for Development of Colorectal Cancer: Act Now

NCT Number: NCT03728348

Document Date: October 16th, 2018

AN EVALUATION OF A MULTI-TARGET STOOL DNA (mt-sDNA) TEST, COLOGUARD, FOR CRC SCREENING IN INDIVIDUALS AGED 45-49 AND AT AVERAGE RISK FOR DEVELOPMENT OF COLORECTAL CANCER: ACT NOW

Investigational plan

Study Title:	An Evaluation of a Multi-target Stool DNA (mt-sDNA) Test, Cologuard, for CRC Screening in Individuals Aged 45-49 and at Average Risk for Development of Colorectal Cancer
Short Study Title:	Act Now
Study Number:	2018-10
Version and Date:	Protocol Version 2.0, October 16, 2018
Study Sponsor:	Exact Sciences 441 Charmany Drive Madison, WI 53719
Sponsor Contact:	[redacted]

INVESTIGATOR SIGNATURE

Protocol Title: An Evaluation of a Multi-target Stool DNA (mt-sDNA) Test, Cologuard, for CRC Screening in Individuals Aged 45-49 and at Average Risk for Development of Colorectal Cancer: Act Now

Protocol Version and Date: Protocol Version 2.0, October 16, 2018

I have read the protocol and agree to:

- Conduct the investigation in accordance with this protocol, ICH GCP E6 (R2), applicable national, state and local laws and regulations, and conditions of approval imposed by the reviewing IRB/EC;
- Supervise all activities involved in this study when study responsibilities are delegated;
- Ensure that the requirements for obtaining informed consent are met;
- Provide sufficient and accurate financial disclosure information and update information if any relevant changes occur during the investigation and for one year following the completion of the study;
- Maintain the confidentiality of all information received or developed in connection with this protocol;
- Adhere to the publication policy of Exact Sciences for data collected during this trial;
- Not initiate this study without approval from the appropriate Institutional Review Board (IRB)/Ethics Committee (EC) and understand that any changes to the protocol must be approved in writing by the Sponsor and the IRB/EC before they can be implemented, except where necessary to eliminate immediate risks to the subject.

_____ _____

Signature of Principal Investigator Date

_____

Printed Name of Principal Investigator

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1 LIST OF ABBREVIATIONS

Abbreviation	Definition
AA	Advanced Adenoma
AE	Adverse Event
CRC	Colorectal Cancer
CRF	Case Report Form
CUC	Chronic Ulcerative Colitis
DNA	Deoxyribonucleic Acid
EC	Ethics Committee
FAP	Familial Adenomatous Polyposis
FDA	Food and Drug Administration
FIT	Fecal Immunochemical Test
FoBT	Fecal Occult Blood Test
GCP	Good Clinical Practice
IBD	Inflammatory Bowel Disease
ICH	International Council for Harmonization
IRB	Institutional Review Board
IVD	In Vitro Diagnostic
HNPCC	Hereditary Non-Polyposis Colorectal Carcinoma (Lynch Syndrome)
MSTF	Multi Society Task Force
mt-sDNA	Multi-target stool DNA
SAE	Serious Adverse Event
UADE	Unanticipated Adverse Device Effect
USPSTF	United States Preventive Services Task Force

2 DISCLOSURE STATEMENT

This document contains information that is confidential and proprietary to Exact Sciences. This information is being provided to you solely for the purpose of evaluating and/or conducting a clinical study for the Sponsor. You may disclose the contents of this document only to study personnel under your supervision, institutional review boards (IRBs)/ ethics committees (ECs), or duly authorized representatives of regulatory agencies for this purpose under the condition that they maintain confidentiality. The contents of this document may not be used in any other clinical study, disclosed to any other person or entity, or published without the prior written permission of the Sponsor. The foregoing shall not apply to disclosure required by any regulations; however, you will give prompt notice to the Sponsor of any such disclosure. All other nonpublic information provided by the Sponsor, as well as any information that may be added to this document, also is confidential and proprietary to the Sponsor and must be kept in confidence in the same manner as the contents of this document.

3 PROTOCOL SYNOPSIS

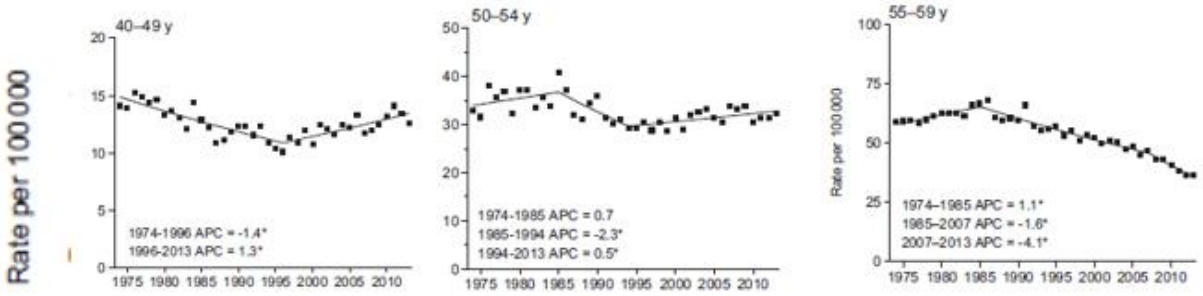
Primary Objective:	The primary objective is to confirm the specificity of the multi-target stool DNA test (mt-sDNA), Cologuard, in an average risk population, ages 45-49.
Study Design:	Prospective, cross-sectional, multi-center study
Investigational Device	Multi-target stool DNA test (mt-sDNA), Cologuard, (Exact Sciences, Madison, WI)
Population:	Subjects 45-49 years of age who are at average risk for development of CRC. 942 subjects are targeted to enroll.
Primary Endpoint:	The primary endpoint of this study is specificity of the mt-sDNA test (Cologuard) in the 45-49 age group in an average risk population, using colonoscopy with histopathology (when required) as the reference method. The primary analysis requires the one-sided 97.5% lower bound of the specificity of Cologuard to exceed 85.0%.
Study Duration:	Duration of study participation for each subject will be approximately 60 days from enrollment into the study.
Study Procedures:	Subjects providing written informed consent to participate in the study will complete the mt-sDNA test (Cologuard) followed by a screening colonoscopy which should be completed within approximately 60 days of enrollment. Subjects may also choose to consent to 2018-10B and provide a blood sample at the time of enrollment.

4 BACKGROUND / RATIONALE

4.1 Background

The 2018 American Cancer Society colorectal cancer (CRC) screening guideline [1] now recommends initiating colorectal cancer (CRC) screening at age 45 in average risk individuals. This change in a prominent national guideline was based on recent data [2] showing that the incidence of CRC in the 40-49 age group has increased with that of the 45-49-year-old group similar to that of 50-54-year-olds. There has been a 51% increase in the incidence of CRC from 1994 to 2014 and an 11% increase in mortality from 2005 to 2015 among individuals 55 years old and younger. Importantly, this change appears to be a birth cohort effect, likely starting for those born in the early 1950s who will carry this increased risk forward for their lifetimes. Compared to birth cohorts between 1890-1950 where the rate of CRC has been steadily declining, the rate in those generations born after 1950 has been steadily increasing. The ACS estimates that in 2014, over 7,073 cases of CRC were diagnosed in individuals aged 45 to 49 years with over 16,450 cases diagnosed in patients under 50.

Rising incidence of CRC in 40-49-year-olds compared to decreasing incidence in those 50 years of age and older (from Siegel R et al JNCI 2017 [2]).



Previously, a recommendation for starting at age 45 in average risk individuals was proposed by the 2009 American College of Gastroenterology guideline in African Americans [3] and reiterated in the 2017 U.S. Multi-Society Task Force CRC screening guideline [4]. The USMSTF includes members of the American Gastroenterological Association, the American College of Gastroenterology, and the American Society of Gastrointestinal Endoscopy. While the 2016 United States Preventive Services Task Force guideline did not recommend starting screening at age 45 [5] and continued to give its “A” rating to screening average risk individuals from ages 50-75, the data supporting a cohort effect generally showing increased risk in younger individuals were not available when the USPSTF reviewed the CRC screening evidence base prior to the 2016 guideline update.

According to Dr. Thomas Weber, former Chair of the National Colorectal Cancer Roundtable and director of surgical oncology for the northwest region of Northwell Health, as quoted in the N.Y Times [6], “Solid epidemiological data from our national cancer registries documents a dramatic increase in the incidence of colon and especially rectal cancer among individuals under the age of 50, and the vast majority of those cases are in the 40- to 49-year-old age bracket.”

Importantly, the ACS guideline includes the multi-target stool DNA test as one of the recommended methods for CRC screening, including for the expanded age range of 45-49 years of age. The ACS Guideline development group states, “The GDG concluded that mt-sDNA warrants inclusion among test options based on its sensitivity for detecting CRC, its improved advanced adenoma and serrated sessile polyp detection compared with FIT, and evidence indicating that some adults would choose screening

with mt-sDNA over other CRC screening tests."[7] Currently the labeled indication for mt-sDNA includes individuals 50 years of age and older who are at typical average risk for CRC. In order to accommodate this change in medical practice, Exact Sciences wishes to expand the labeled indication for mt-sDNA into this 45-49 age group.

4.2 Rationale

Given its high sensitivity for CRC and advanced pre-cancers demonstrated in the mt-s DNA pivotal study [8] and given the safety of mt-sDNA as a non-invasive stool-based test, mt-sDNA could be an important option for patients and providers in this younger age group. [redacted]

The study is designed to provide additional evidence of test performance in this age group to support an application for FDA label expansion. Biologically, there is little reason to believe there is a significant difference in the underlying mechanism of CRC in average risk individuals age 45-49 vs. 50-59, which is included in the current label.

5 OBJECTIVES

5.1 Primary Objective

The primary objective is to confirm the specificity of the mt-sDNA test (Cologuard) in an average risk population, ages 45-49.

6 ENDPOINTS

6.1 Primary Endpoint

The primary endpoint of this study is specificity of the mt-sDNA test (Cologuard) in the 45-49 age group in an average risk population, using colonoscopy with histopathology (when required) as the reference method. The primary analysis requires the one-sided 97.5% lower bound of the specificity of Cologuard to exceed 85.0%.

6.2 Other Outcomes

Other outcomes to be assessed include the following

- Sensitivity for CRC + advanced adenoma
- The positive and negative predictive values
- The positive and negative likelihood ratios (PLR and NLR, respectively)
- The distribution of colorectal epithelial lesions (by Category) among positive mt-sDNA test (Cologuard) subjects
- The rate of no mt-sDNA (Cologuard) result

7 STUDY DESIGN

This is a prospective, cross-sectional, multi-center study to confirm the specificity of the mt-sDNA test (Cologuard) in average risk individuals ages 45 to 49.

All subjects enrolled into the study will be provided a stool collection kit for the mt-sDNA test (Cologuard) and scheduled for a screening colonoscopy. Stool sample collection must occur prior to subjects initiating bowel preparation for the colonoscopy procedure. All subjects will undergo a screening colonoscopy within approximately 60 days of enrollment.

The results of the mt-sDNA test (Cologuard) will be compared with the results of the colonoscopy and with any corresponding diagnostic histopathology results from biopsies or excised tissues obtained for clinical purposes to confirm the performance estimates.

Stool samples collected by the subject for the mt-sDNA test (Cologuard) will be sent to Exact Sciences Laboratories and tested. Individuals conducting the mt-sDNA tests will be blinded to all subject clinical information, including colonoscopy results. Investigators, subjects, and endoscopists will be blinded to the mt-sDNA test (Cologuard) results, and the results of the tests will not be used for clinical management of study subjects.

All subjects enrolled in the study will also be offered the opportunity to provide a blood sample for biomarker evaluation and assay optimization (Protocol 2018-10B). Blood samples will be used for research on biomarkers from plasma and serum.

8 STUDY DEVICE

8.1 Description of the Investigational Device

The mt-sDNA-based test (Cologuard) is a colorectal cancer screening option that consists of a stool collection kit and proprietary assay methodologies for the detection of fecal hemoglobin and fecal DNA markers known to be associated with CRC [redacted] Cologuard is approved by the FDA for use in average risk adults ages 50 years or older. Cologuard has not been evaluated in average risk individuals under the age of 50.

The Stool Collection Kit includes: Sample Collection Instructions, a stool collection container and support bracket, a bottle of liquid sDNA preservative, a fecal hemoglobin sample tube, return shipping box, and return shipping label. The stool collection kits will be labeled as investigational use only. The stool collection kits will be provided by the Sponsor. The sites are responsible for management of the stool collection kits. The investigator must maintain complete and current accountability records (including kit receipt, dispensation, and return) for all kits supplied by the Sponsor. All unused kits and their original packaging should be kept on-site for reconciliation by a site monitor. The site must return all unused kits and packaging after accountability has been performed at the end of the study. Stool collection kit return must be documented in the accountability records.

8.2 Regulatory History

Cologuard received Pre-Market Approval from the U.S. Food & Drug Administration on August 11, 2014 (P130017) (as well as approval for subsequent PMA supplements) with the following Indication for use:

Cologuard is intended for the qualitative detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of CRC or AA and should be followed by diagnostic colonoscopy. Cologuard is indicated to screen adults of either sex, 50 years or older, who are at typical average-risk for CRC. Cologuard is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high risk individuals.

9 SUBJECT SELECTION AND WITHDRAWAL

942 subjects are targeted to enroll. The following eligibility criteria are designed to select subjects who are at average risk for development of CRC. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. No waivers to these criteria will be permitted.

9.1 Inclusion Criteria

Subjects must meet the following criteria to be eligible for the study:

1. Subject is at average risk for development of CRC.
2. Subject is able and willing to undergo a screening colonoscopy.
3. Subject is ≥ 45 and ≤ 49 years of age at the time of enrollment.
4. Subject is willing and able to sign informed consent.
5. Subject is able and willing to provide stool sample(s) according to written instructions provided.

9.2 Exclusion Criteria

Subjects presenting with any of the following will not be included in the study:

1. Subject has a history of CRC or adenoma.
2. Subject has ≥2 first-degree relatives who have been diagnosed with CRC
3. Subject has one first-degree relative with CRC diagnosed before the age of 60.
4. Subject has any of the following:
    - Overt rectal bleeding, e.g., hematochezia or melena within the previous 30 days (blood on toilet paper, after wiping, does not constitute rectal bleeding).
    - Positive fecal occult blood test or FIT within the previous six (6) months.
    - Subject has had a previous colonoscopy.
    - Subject has undergone any double-contrast barium enema, virtual (CT-based) colonoscopy, or flexible sigmoidoscopy within the previous five (5) years.
5. Subject has a diagnosis or personal history of any of the following conditions, including:
    - Familial adenomatous polyposis (also referred to as "FAP", including attenuated FAP and Gardner's syndrome).
    - Hereditary non-polyposis CRC syndrome (also referred to as "HNPCC" or "Lynch Syndrome").
    - Other hereditary cancer syndromes including but are not limited to Peutz–Jeghers Syndrome, MYH-Associated Polyposis (MAP), Turcot's (or Crail's) Syndrome, Cowden's Syndrome, Juvenile Polyposis, Neurofibromatosis and Familial Hyperplastic Polyposis.
6. Subject has a family history of:
    - Familial adenomatous polyposis (also referred to as "FAP").
    - Hereditary non-polyposis CRC syndrome (also referred to as "HNPCC" or "Lynch Syndrome").
7. Subjects with Cronkhite-Canada Syndrome.
8. Subject has a diagnosis of inflammatory bowel disease (IBD) including chronic ulcerative colitis (CUC) and Crohn's disease.

9. Subject has a history of aerodigestive tract cancer.
10. Subject has had a prior colorectal resection for any reason other than sigmoid diverticular disease.
11. Subject has any condition that in the opinion of the investigator should preclude participation in the study.

9.3 Subject Withdrawal

Subjects may be withdrawn from the study by any of the following mechanisms:

1. Voluntary withdrawal of consent to participate by the subject at any time during the study.
2. Determination by the clinical investigator that it is in the best interest of the subject.
3. Determination by the Sponsor it is in the best interest of the subject or study.
4. Stool sample not provided within 30 days of enrollment.
5. Colonoscopy procedure is not done.

All data available from enrollment to subject withdrawal will be collected unless the subject withdraws consent from the study. Data for all study visits will be collected for subjects who discontinue participation but who do not withdraw consent. Withdrawal of consent must be documented by site personnel in the subject source record.

9.4 Subject Replacement

Enrolled subjects may be replaced for any of the following reasons:

1. No stool sample provided and/or no completed colonoscopy procedure.
2. A valid stool sample result cannot be obtained for a subject after two (2) attempts (Section 10.4).

10 STUDY PROCEDURES

10.1 Overview

Individuals ages 45 to 49 who are at average risk for development of CRC will be invited to participate in the study. 942 subjects are targeted to enroll. Subjects providing written informed consent to participate in the study will complete the mt-sDNA test (Cologuard) followed by a screening colonoscopy which should be completed within approximately 60 days of enrollment. Subjects may also choose to consent to 2018-10B and provide a blood sample at the time of enrollment.

Refer to Appendix 1.0 – Study Schema and Appendix 2.0 – Schedule of Activities

10.2 Enrollment

Subjects are considered enrolled once written informed consent is obtained and subject eligibility confirmed.

10.3 Blood Sample Collection

Subjects enrolled in this study should also be offered enrollment in an optional blood collection study (2018-10B). If the subject also consents to 2018-10B, approximately 40 mL of peripheral blood will be collected from the subject. Blood sample collection should occur on the same day as enrollment.

10.4 Stool Sample Collection

Enrolled subjects will complete the mt-sDNA test (Cologuard) prior to bowel preparation for the colonoscopy procedure. Subjects enrolled into the study will be provided with a Stool Collection Kit. Stool samples must be collected prior to initiation of bowel preparation for the colonoscopy procedure. Stool samples will be collected by following the instructions provided. Instructions for kit return are provided to the subject with the Stool Collection Kit.

The shipment and arrival of the Stool Collection Kit at Exact Sciences Laboratories will be tracked. Once the sample arrives at Exact Sciences Laboratories, it will be inspected for acceptability per the specifications outlined in the Cologuard Instructions for Use. If the stool sample is not collected according to the instructions for use or damaged in a way that compromises the specimen, a repeat sample collection may be requested if the collection can occur prior to the initiation of bowel preparation for the scheduled colonoscopy. The site will be asked to contact the subject to request another stool specimen. At that time, another stool collection kit will be dispensed to the subject by the site.

10.5 Sample Assays

Assay results will be recorded as 'Positive', 'Negative', 'Sample Could Not Be Processed', or 'No Result Obtained.' If there are no valid results, a repeat test may be requested. Repeat stool sample requests will be sent to the site. Repeat stool sample collection must occur prior to bowel preparation for the colonoscopy procedure. If a valid stool sample result cannot be obtained for a subject after two (2) attempts, the subject will be withdrawn and may be replaced.

The mt-sDNA test (Cologuard) result will not be provided to investigators for clinical management of study subjects. All mt-sDNA test results will remain blinded to investigators and study subjects.

10.6 Colonoscopy and Histopathology

Every subject will undergo a screening colonoscopy after completing a mt-sDNA test (Cologuard) test. Colonoscopies should occur as soon as possible following informed consent and stool collection. Subjects who have poor bowel preparation and will not repeat the procedure or subjects who do not complete a colonoscopy should be withdrawn and may be replaced. Bowel preparation procedures and colonoscopy will be performed following the usual practice at each clinical site. Personnel performing the colonoscopy and producing the resulting report and the personnel performing the histopathological review of tissue will remain blinded to the results of the mt-sDNA test (Cologuard) result.

10.6.1 Colonoscopy Procedure

Bowel preparation procedures and colonoscopy will be performed following standard of care at each clinical site. The colonoscopist will record the quality of the bowel preparation (after washing/suctioning) as follows:

- **Excellent**: minimal fecal residue and liquid, small volume of liquid easily aspirated, <5% of colonic surface covered;
- **Good**: small amounts of fecal residue, volume of clear liquid covering 5-25% of surface, easily aspirated;
- **Fair**: moderate amounts of fecal residue; stool limited examination but >90% could be examined; or
- **Poor**: large amounts of stool, <90% of surface could be examined.

Cecal intubation will be documented with photographic evidence and/or documentation of cecal intubation; and colonoscope withdrawal time will be recorded. Withdrawal time is defined as the time from cecal identification to the time when the colonoscope is withdrawn across the anus (Barclay, Vicari, Doughty, *et al*, 2006.).

A bowel preparation considered excellent, good, or fair will be acceptable. Subjects with no findings on the colonoscopy, and no photographic evidence of cecal intubation or no documentation of cecal intubation in the report, will be deemed to have a failed colonoscopy. These subjects will be deemed to be unevaluable and therefore will be excluded from analysis of the primary and secondary endpoints.

Those subjects determined to have had a poor bowel preparation (after washing/suctioning) may repeat the colonoscopy again if the Investigator feels this is in the best interest for the subject. The subject may repeat the colonoscopy procedure. A repeat stool specimen is not required.

A completed colonoscopy procedure will be considered and defined as: reaching the cecum (or) reaching the junction between the small and large intestine (in the event the cecum has been resected).

A colonoscopy wherein a CRC or AA was identified will be included in the analyses regardless of any limiting factors experienced during the colonoscopy.

10.6.2 Colonoscopy and Histopathology Reports

Colonoscopy findings will be recorded on the colonoscopy reports per site-specific standard of practice. Histopathology interpretation of tissue samples will be conducted according to each of the clinical site's local histopathology practice provider(s) as used for daily patient management.

Subjects who have lesions identified by colonoscopy that are neither biopsied initially nor excised subsequently or that are lost during lesion retrieval and, therefore, not submitted for histopathologic analysis, will be identified by the sites, and the colonoscopy report will be sent to the study central pathologist(s) for review. Because of the absence of confirmatory histopathologic diagnosis, these subjects will be excluded from the study based on the fact that they cannot be categorized for the study analysis.

The sites will grant independent central pathologist(s) access to colonoscopy, surgical, and histopathological reports by providing them a copy of those reports. All protected health information on the documents will be removed before release to the central pathologist(s). The study-specific subject identification number will be used to identify colonoscopy reports and applicable histopathology reports submitted to the central pathologist(s).

Histopathological results will be categorized as defined in **Appendix 4**. For subjects that undergo surgical removal of CRC and/or non-CRC lesions, an excisional surgical pathology report should be available. To ensure diagnostic accuracy, investigative sites with subjects diagnosed with CRCs or AAs may be required to provide representative histology slides for review by a central pathologist. When the central pathologist histopathology result differs from the local histopathology result, the results will be adjudicated with a review and interpretation by a second central pathologist.

Patient management will be conducted locally at each investigative site based upon colonoscopy outcomes and the histopathology results reported by the local pathology lab. In the event that the centralized histopathology laboratory assigns a higher-grade histology result than the local histopathology laboratory, the Investigator will be notified. The Investigator should maintain the central pathology results

with the subject's source records. The central pathology laboratory results may not be available prior to the subsequent treatment of the subject.

10.7 Data Collection

Subject data will be collected on CRFs and provided to the Sponsor. After subjects have provided informed consent, demographics, medical history, social history, and family history will be obtained. The clinical site will send a copy of available colonoscopy reports and applicable histopathology reports and/or other diagnostic information to the Sponsor. All reports that are provided to the Sponsor will be de-identified of all protected health information, prior to submission to the Sponsor.

10.8 End of Study

Subjects will be considered completed from the study when they have provided a stool sample and have completed their colonoscopy procedure, OR at the point of subject withdrawal per Section 9.3.

Ensure all data is assessed, entered into the CRF and reporting on AEs/SAEs, UADEs, and/or Protocol Deviations has occurred, if necessary.

10.9 Sample Retention

Retained samples comprise of residual stool samples. Retained subject samples will not be used other than to develop and evaluate the performance of biomarker assays to detect cancers. Retained samples will be de-identified of all protected health information, and clinical study data will only be associated by a subject identification number. Samples will be stored for no more than 20 years in Exact Sciences' or designee's on-site biorepository and will be used for developing and evaluating the performance of biomarker assays for cancer detection. Specimens de-accessioned from the biorepository will be destroyed under standard biohazardous material protocol and record of the removal and destruction will be maintained for 7 years by the Sponsor. Retained subject samples will not be used for any other purposes.

10.10 Unusable Samples

Subjects who provide stool samples which are insufficient for testing may be asked to provide another stool sample if the subject has not yet begun bowel preparation for colonoscopy.

11 ADVERSE EVENT REPORTING

11.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable or unintended sign, symptom, or disease temporally associated with the use of an investigational product or protocol-imposed intervention, regardless of attribution.

[redacted] The liquid buffer/liquid preservative in the quantities provided is non-toxic. An AE may be investigated as an Unanticipated Adverse Device Effect (UADE), if applicable.

Adverse events commonly associated with colonoscopy will not be collected. Such events include: Abdominal discomfort, bowel irregularity, bleeding, intestinal perforation, and adverse reaction to the sedation.

Surgical intervention, such as, but not limited to, colectomy and sub-mucosal resection are part of routine patient care for subjects with CRC or AA; therefore, adverse events commonly associated with surgeries will not be collected. Such events include: Bleeding, blood clots in the legs (deep vein thrombosis) and lungs (pulmonary embolism); infection; injury to organs near the colon, such as the bladder and small intestines; and tears in the sutures that reconnect the remaining parts of the digestive system.

11.2 Reporting Period

Adverse events will only be recorded for events that occur during or otherwise associated with the subject use of the mt-sDNA test (Cologuard), specifically the stool sample collection procedure. AEs are collected from the time of enrollment to return of the Stool Collection Kit. All AEs will be reported on the adverse event page(s) of the CRF.

11.3 Serious Adverse Events

Serious adverse events are not expected for this study but will be reported to the Sponsor if they occur.

A serious adverse event (SAE) is any AE that results in one or more of the following outcomes.

- Death; Note: “Death” should not be reported as an SAE. The cause of death should be reported as the SAE. The only exception is “Sudden Death” when the cause is unknown.
- A life-threatening illness or injury;
- A permanent impairment of a body structure or a body function;
- Required in-patient hospitalization or prolongation of existing hospitalization;
- Necessitates medical or surgical intervention to prevent permanent impairment of a body structure or a body function; or
- Fetal distress, fetal death or a congenital abnormality or birth defect.

11.4 Unanticipated Adverse Events

An unanticipated adverse device effect (UADE) is “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects” (21 CFR 812.3(s)).

11.5 Reporting Requirements

11.5.1 SAE Reporting Requirements

If a SAE occurs, Exact Sciences or its designee is to be notified within 24 hours of awareness of the event by the Investigator. In particular, if the SAE is fatal or life-threatening, notification to Exact Sciences or its designee must be made immediately, irrespective of the extent of available adverse event information.

This timeframe also applies to additional new information (follow-up) on previously forwarded SAE reports.

In the rare event that the Investigator does not become aware of the occurrence of a SAE immediately (e.g., if an outpatient study subject initially seeks treatment elsewhere), the Investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

For all SAEs, the Investigator is obligated to pursue and provide information to Exact Sciences or its designee in accordance with the timeframes for reporting specified above. In addition, the Investigator may be requested by Exact Sciences or its designee to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the adverse event case report form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Exact Sciences or its designee. SAEs may be reported to the local IRB/EC per institutional requirements.

11.5.2 Non-Serious Adverse Event Reporting Requirements

All AEs will be reported on the adverse event page(s) of the CRF. It should be noted that the form for collection of SAE information is not the same as the adverse event CRF.

11.5.3 Reporting Requirements of Unanticipated Adverse Devices Effects (UADEs)

All UADEs must be reported by the Investigator to the Sponsor within 3 days from identification or awareness of the event. Investigators are responsible for preparing and submitting complete, accurate, and timely reports to the reviewing IRB/EC of any UADE occurring during an investigation as soon as possible, but in no event later than 10 working days after they first learn of the effect.

11.5.4 Sponsor Reporting Requirements to Regulatory Authorities

Exact Sciences will immediately conduct an evaluation of any UADE. If Exact Sciences determines that an UADE presents an unreasonable risk to subjects Exact Sciences shall terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination shall occur no later than 5 working days after the Sponsor makes this determination and not later than 15 working days after the Sponsor first received notice of the effect.

12 DATA ANALYSIS AND STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

12.1 Effectiveness Endpoints

12.1.1 Definitions

Advanced Neoplasia (AN)	Colonoscopic findings that include colorectal cancer (CRC) (category 1) and advanced adenoma (AA) (category 2).
Non-advanced neoplasia (NAN)	Colonoscopic findings ≤ 1.0 cm that exclude colorectal cancer (CRC) and advanced adenoma (AA) and include categories 3 – 5.
Sensitivity (Se)	The sensitivity (Se) or true positive fraction of the test is defined as TP/(TP + FN)
Specificity (Sp)	The specificity (Sp) or true negative fraction of the test is defined as TN/(TN+FP)
False positive fraction (FPF)	The false positive fraction (FPF) is defined as FP/(TN+FP)
Odds ratio	The odds ratio (OR) is defined as (TP/FP)/(FN/TN)
Negative likelihood ratio	The negative likelihood ratio (NLR) is defined as (1-Se)/(Sp)
Positive likelihood ratio	The positive likelihood ratio (PLR) is defined as Se/(1-Sp)
Negative Predictive Value	The Negative Predictive Value (NPV) is defined as: TN/(FN+TN) and/or [Sp*(1-Prevalence)]/[(1-Se)*(Prevalence+Sp*(1-Prevalence)].
Positive Predictive Value	The Positive Predictive Value (PPV) is defined as: TP/(TP+FP) and/or (Se*Prevalence)/[Se*Prevalence+(1-Sp)*(1-Prevalence)]

12.2 Primary Endpoint

The primary endpoint of this study is the specificity of the mt-sDNA test (Cologuard) in the 45-49 age group in an average risk population, using colonoscopy with histopathology (when required) as the reference method. The primary analysis requires the one-sided 97.5% lower bound of the specificity of Cologuard to exceed 85.0%.

The primary endpoint of this study is to confirm the specificity of the mt-sDNA test (Cologuard) in the 45-49 age group in an average risk population. Specificity will be determined by comparing the mt-sDNA test result to the results of optical colonoscopic examination and confirmation of the colonoscopic findings by histopathology, if appropriate.

Possible categories of colonoscopic findings are as follows:

1. CRC, all stages (I-IV);
2. Advanced adenoma, including the following subcategories:
   - 2.1 Adenoma with carcinoma *in situ* or high-grade dysplasia, any size
   - 2.2 Adenoma, villous growth pattern (≥ 25%), any size
   - 2.3 Adenoma ≥ 10 mm in size, or
   - 2.4 serrated lesion, ≥ 10 mm in size;
3. 1 or 2 adenoma(s), > 5 mm in size, or < 10 mm size, non-advanced;
4. ≥ 3 adenomas, < 10 mm in size, non-advanced;
5. 1 or 2 adenoma(s), ≤ 5 mm in size, non-advanced;
6. Negative – no neoplastic findings, including the following subcategories:
   - 6.1 Negative, no colorectal neoplasia upon histopathological review
   - 6.2 No findings on colonoscopy, no biopsy(s) taken

X. Index lesion cannot be categorized

Subjects with colonoscopic findings in categories 3-6 above will be included in the specificity analysis as negative outcomes for the primary endpoint calculation. Thus, specificity to advanced neoplasia (AN) will be calculated for the primary endpoint evaluation. The colonoscopically 'negative' population (category 6) will include those subjects with a colonoscopy showing no findings of colorectal neoplasia, non-neoplastic disease, and/or hyperplastic polyps ≤ 10 mm in diameter.

Subjects with no findings on the colonoscopy, no photographic evidence of cecal intubation and no documentation of cecal intubation in the report, will be deemed to be unevaluable and therefore will be excluded from all analyses. Subjects found to have occult inflammatory bowel disease (IBD) on colonoscopy will be excluded from all analyses. Subjects with unevaluable Exact CRC screening test results will be excluded from the primary endpoint analyses. All excluded cases will be assessed for bias.

Subjects with colonoscopic/histopathologic findings in category 1 (confirmed colorectal cancer cases) will be considered to have a positive outcome and will be included in the CRC sensitivity calculations. Note: Due to the low prevalence of CRC in the Age 45-49 group, there will likely be no CRC prospectively enrolled in the study, so no endpoints will be designed around CRC sensitivity. Similarly, due to the low prevalence of AA in the Age 45-49 group there may only be approximately 29 subjects* with colonoscopic/histopathologic findings in category 2 (advanced adenomas) prospectively enrolled in the study, so no endpoints will be designed around AA sensitivity.

*Advanced adenoma prevalence among never-screened individuals aged 45-49 years may be derived, based on simulation results, to be 3.92%. 731*3.92%= 29 [9].

12.3 Sample Size and Rule Out Lower Threshold Justification

The minimum number of non-advanced neoplasia and negative instances (categories 3-6.2) needed to rule out an 85.0% lower bound on the specificity with 90% power for a one-sided test with 2.5% Type 1 error according to an exact binomial distribution is 225, as outlined in Table 1. Furthermore, the minimum sample size needed such that the confidence interval width for the alternative proportion (92.2%) is within +/-2.2% (i.e. 92.2%+/-2.2% = 90.0%/94.4%) is 731. Using this sample size, the power of the study is >99.99%.

Table 1: One-sided Exact Test Power: Single Proportion

Sample Size Scenarios	1	2
Test significance level, α	0.025	0.025
One or 2-sided test	1	1
Null hypothesis proportion	85.0%	85.0%
Alternative Proportion	92.2%	92.2%
Power (%)	90%	>99.99%
# Non-CRC/AA Cases	225	731

The alternative proportion of 92.2% is the observed specificity in the DeeP-C study [SSED P130017, Source: fda.gov] for the age group 50-59. It is anticipated that the specificity of Cologuard in individuals age 45-49 is not different than the specificity of the next closest age category. The null hypothesis proportion of 85.0% is identical to specificity null hypothesis used in the DeeP-C study (NCT).

A minimum sample size of 193 evaluable subjects (age 45-49 inclusive) is required to adequately power the primary analysis. This minimum sample size requirement will be adjusted to 731 to more accurately estimate the specificity by narrowing the CI. The total enrollment target is adjusted to 942 to account for a 3% prevalence of advance neoplasia and a 20% drop out rate.

12.3.1 Analysis Approach, Primary Effectiveness Analysis

Primary Effectiveness Analysis:

The primary analysis for mt-sDNA test (Cologuard) specificity will be performed using an exact binomial (Clopper-Pearson) test to compute the one-sided 97.5% lower bound; the lower bound must exceed 85.0%. Specificity will be assessed for a random sample of 731 colonoscopy negatives.
Rejecting the null hypothesis will demonstrate the Specificity in the Age 45-49 group is no worse than the pre-specified lower bound limit established in the DeeP-C protocol.

12.4 Other Prespecified Analyses

- Analysis of distribution of test performance by race/ethnicity using chi-square tests.
- Analysis of distribution of test performance by age (yearly interval, 45-49) using chi-square tests.
- Analysis of test performance by gender using chi-square tests.
- Analysis of test performance by polyp size and location (including those with no polyps). This model will be repeated to include gender, race, and age. Modeling will be regression-based.
- Analysis of Sensitivity to Advance Adenoma (Clopper-Pearson Exact confidence interval). Due to the expected low frequency of Advanced Adenoma, subgroup (Category 2.1, 2.2, 2.3, 2.4) analysis will not be performed, but observed rates will be reported.
- Analysis of Specificity inclusive and exclusive of non-advanced neoplasia (Categories 3.0-5.0) (Clopper-Pearson Exact confidence interval).

12.4.1 Additional Analysis

- NPV, PPV, Odds Ratio, NLR, PLR and diagnostic yield for the 45-49 age group will be estimated using the observed study specificity and different assumptions regarding prevalence of CRC/AA and assumptions regarding sensitivity to CRC/AA.

12.5 Handling of Missing Data

The primary analysis will be based on all available data without imputation for missing data. Missing data on mt-sDNA test (Cologuard) outcome and CRC/AA occurrence will be imputed using other available observed study data. Based on exclusion patterns, demographic data such as age, gender, race, and ethnicity will be considered for imputing missing data. Detailed imputation methods to be used will be documented in the SAP. The method of Campbell, Pennello, and Yue will be used to assess the impact of missing cases [10].

12.6 General Statistical Methods

All tables, listings and figures will be produced using SAS version 9.2 (or higher). Data analysis will be performed using SAS: Statistical Analysis Software (SAS 9.2 and JMP Version 9.0.3 or higher, both from SAS Institute, Cary, and NC). The testing results from all sites will be compiled and data analysis will be performed on the compiled results as described in this protocol.

12.6.1 Categorical Variables

Categorical variables will be summarized by presenting the number and percent of observations in each category and the two-sided 95% confidence interval (CI), unless otherwise noted. When calculating percentages, the denominator will be the number of participants in the entire study population, unless otherwise specified in the output. Comparisons across Categorical Subgroups will be performed using Chi-Square tests.

12.6.2 Continuous Variables

Continuous variables will be summarized using n (sample size), mean, SD, minimum and maximum values, and 95% confidence interval unless otherwise noted. Continuous variable data distributions will be evaluated for Normality using Shapiro-Wilk method using 99% confidence level method. Distribution-free descriptive statistics (e.g. median value) would be documented if the Normality test fails.

12.6.3 Determination of Diagnostic Accuracy

These data will be presented in the form of 2 x 2 tables, which will be presented separately for colorectal cancer (CRC), Advanced adenoma (AA), and advanced colorectal neoplasia (CRC+AA). Point estimates of sensitivity and specificity, along with Clopper-Pearson confidence intervals will be used to assess performance against study endpoint.

Results of study will be displayed in 2x2 tables (for CRC, AA, and combined) as shown below:

Table 2: Outcome for all CRC (AA, or combined) subjects

EXACT Test	Reference Test (Colonoscopy)	
	CRC+	CRC-
sDNA +	A (TP)	B (FP)
sDNA -	C (FN)	D (TN)

The table includes the number of true positives (TP: those that have the disease and test positive), false positives (FP: those that do not have the disease and test positive), false negatives (FN: those that do have the disease and test negative) and true negatives (TN: those that do not have the disease and test negative).

13 QUALITY CONTROL AND QUALITY ASSURANCE

During study conduct, Exact Sciences or its agent will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The site monitors may review source documents to confirm that the data recorded on CRFs is accurate. The Investigator and institution will allow site monitors and appropriate regulatory authorities direct access to source documents to perform this verification.

The study site may be subject to review by the (IRB)/Ethics Committee (EC), and/or to quality assurance audits performed by Exact Sciences, or companies working with or on behalf of Exact Sciences, and/or to inspection by appropriate regulatory authorities.

It is important that the Investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

14 DATA HANDLING AND RECORD KEEPING

All references to the Sponsor in this section include all designees e.g., Contract Research Organizations or Consultants acting on behalf of the Sponsor.

14.1 Protocol Deviations

The site should make all efforts not to deviate from any of the study procedures or requirements as described in this protocol, except where necessary to eliminate immediate risks to the subject(s).

Any deviations from this protocol must be reported to the Sponsor and documented properly. Protocol deviations must also be reported to the IRB/EC per IRB/EC policy.

14.2 Direct Access to Source Data/Documents

The Investigator's institutions must permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source data/documents. This can include Electronic Medical Records (EMR) systems and/or original paper records.

14.3 Case Report Forms (CRFs)

As used in this protocol, the term Case Report Form (CRF) should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each enrolled subject. The completed original CRFs are the sole property of Exact Sciences and should not be made available in any form to third parties, except for authorized representatives of Exact Sciences or appropriate regulatory authorities, without written permission from Exact Sciences.

The Investigator has ultimate responsibility for the collection and reporting of all clinical, safety and laboratory data entered on the CRFs, any other data collection forms and source documents, ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when required. The CRFs must be signed by the Investigator to attest that the data contained on the CRFs is true. Any corrections to entries made in the CRFs or source documents must be dated, initialed and explained (if necessary) and should not obscure the original entry.

14.4 Records Retention

The ICH E6(R2) Good Clinical Practice: Consolidated Guidance defines source documents as, "Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial)."

To enable evaluations and/or audits by regulatory authorities or Exact Sciences, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, e.g., CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, serious adverse event forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The

Investigator or Sponsor shall maintain study records for a period of 2 years after the latter of the following three dates: the date on which the investigation is terminated or completed, the date that the records are no longer required for purposes of supporting a premarket approval application or a notice of completion of a product development protocol, or date provided in applicable national law.

If the Investigator becomes unable for any reason to continue to retain study records for the required period (e.g., retired, relocation), Exact Sciences should be prospectively notified. The study records must be transferred to an appropriately qualified designee acceptable to Exact Sciences, such as another Investigator, another institution, or to an independent third party arranged by Exact Sciences. The Investigator must obtain Exact Sciences' written permission before disposing of any records, even if retention requirements have been met.

15 ETHICS

15.1 Risk Analysis

15.1.1 Risk to the subject

The risks of using the mt-sDNA test (Cologuard) are similar to the risks associated with any in vitro diagnostic (IVD) assay performed on stool samples. Stool sample collection is performed by the subject in his/her home. It is not anticipated that this collection process will present any significant risk to the subject. The mt-sDNA test (Cologuard) results will not be provided to the investigator or the subject. However, minor risks may be associated with use of the collection kit, as detailed in Section 11.

15.1.2 Benefit to Subject

There are no benefits to the subjects enrolled in this study. The results of the mt-sDNA test (Cologuard) will not be used in the subject's clinical management and both the subject and the Investigator will remain blinded to the results. Only the colonoscopy procedure results will be used for diagnosis and treatment as per the standard of care for the average risk study population.

15.2 Institutional Review Board (IRB) / Ethics Committee (EC)

It is the responsibility of the Investigator to have prospective approval of the study protocol, protocol amendments, informed consent forms, and other relevant documents, e.g., recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the Investigator File. Copies of IRB/EC approvals should be forwarded to Exact Sciences or designee.

15.3 Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), the Declaration of Helsinki (World Medical Association), and applicable local regulatory requirements and laws.

15.4 Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names on any Sponsor forms, reports, publications, or in any other disclosures, except where required by laws. In case of data transfer, Exact Sciences will maintain high standards of confidentiality and protection of subject personal data.

The informed consent form must be in compliance with ICH GCP, local regulatory requirements, and legal requirements.

The informed consent form used in this study, and any changes made during the course of the study, must be prospectively approved by both the IRB/EC and Exact Sciences before use.

The Investigator must ensure that each study subject, or his/her legally acceptable representative, is fully informed about the nature and objectives of the study and possible risks associated with participation. The Investigator, or a person designated by the Investigator, will obtain written informed consent from each subject or the subject's legally acceptable representative before any study-specific activity is performed. The Investigator will retain the original of each subject's signed consent form.

15.5 Reporting of Safety Issues and Serious Breaches of the Protocol or GCP

In the event of any prohibition or restriction imposed (i.e., clinical hold) by an applicable National Authority in any area of the world, or if the Investigator is aware of any new information which might influence the evaluation of the benefits and risks of the investigational product, Exact Sciences should be informed immediately.

16 A DEFINITION OF END TRIAL

End of Trial is defined when 731 subjects have completed required study procedures with complete data receipt or on the date of early termination notice.

17 SPONSOR DISCONTINUATION CRITERIA

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/EC, or at the discretion of Exact Sciences. In addition, Exact Sciences retains the right to discontinue development of the mt-sDNA test (Cologuard) for an average risk population at any time.

If the study is prematurely terminated or discontinued, Exact Sciences will promptly notify the Investigator. After notification, within 30 days, the Investigator must contact all participating subjects who have been enrolled but have not yet been completed or withdrawn. As directed by Exact Sciences, all study materials must be collected and all CRFs completed to the greatest extent possible.

18 AUDITING PROCEDURES

Investigational sites may undergo a quality assurance audit. The Sponsor, an authorized designee or a regulatory agency may conduct the audit. If a regulatory agency requests an audit of the site, the Investigator is required to inform the Sponsor immediately.

19 REFERENCES

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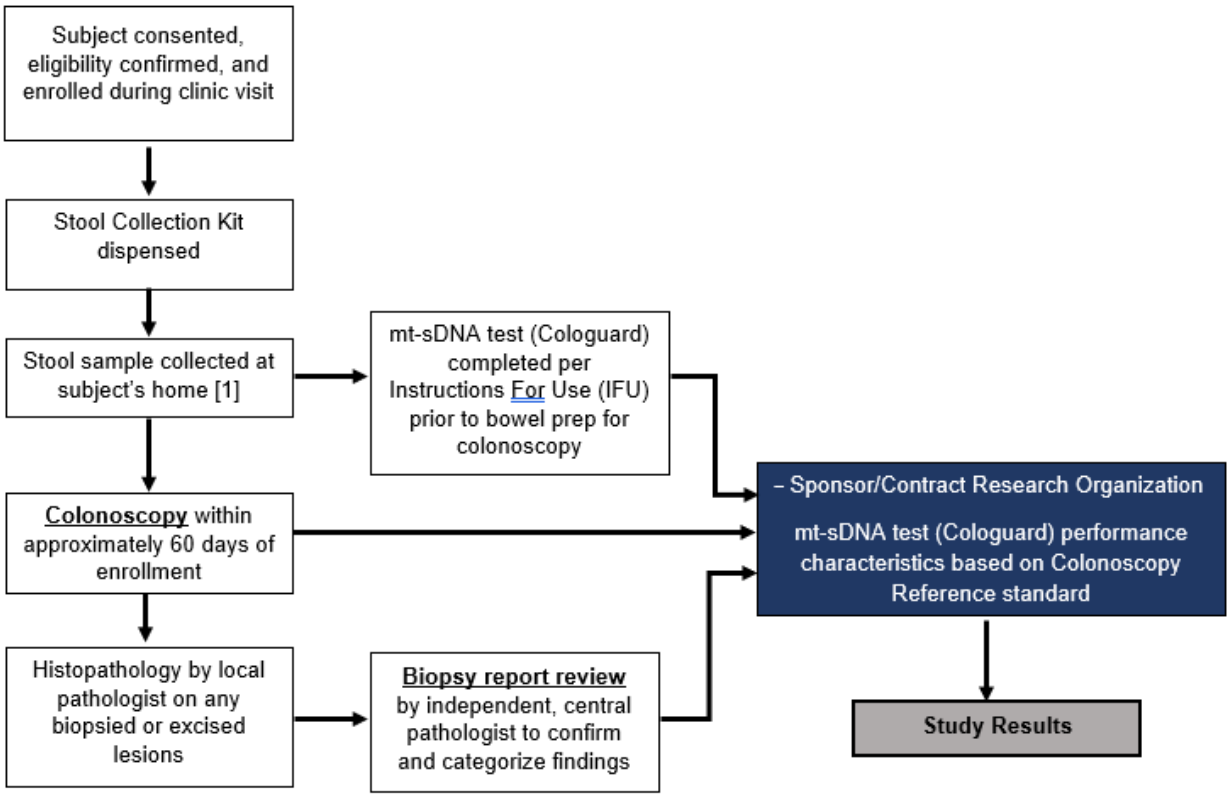
7. Wolf et al, 2018, p. 18; citing: Schroy PC 3rd, Lal S, Glick JT, Robinson PA, Zamor P, Heeren TC. Patient preferences for colorectal cancer screening: how does stool DNA testing fare? Am J Manag Care. 2007; 13:393-400.

8. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. Multitarget Stool DNA Testing for Colorectal-Cancer Screening, New Engl J Med 2014, 370 (14):1287-1297 and supplement

9. Rutter C, Miglioretti D, Savarino J, Bayesian Calibration of Microsimulation Models. J Am Stat Association 2009, 104(488): 1338-1350.

10. Campbell G; Pennello G; Yue L. Missing data in the regulation of medical devices. J Biopharm Stat. 2011; 21(2):180-95 (ISSN: 1520-5711

APPENDIX 1.0 – STUDY SCHEMA



Footnotes for Study Schema

1. Subjects with an unusable stool sample may be dispensed an additional Stool Collection Kit and repeat the stool collection process.

APPENDIX 2.0 – STUDY SCHEDULE

Activity	Enrollment	Stool Sample Collection	Colonoscopy Visit	Colonoscopy and Histopathology Reports	End of Study / Withdrawal
Informed Consent [1]	X				
Medical History and Demographics	X				
Enrollment	X				
Obtain Stool Sample mt-sDNA test (Cologuard)		X			
Colonoscopy Procedure [2]			X		
Submission of all diagnostic reports				X	
Adverse Event Reporting [3]		X			
Discontinuation [4]					X

Footnotes for Study Schedule

1. Informed Consent: Must occur prior to undergoing any study specific procedure
2. Colonoscopy should occur within approximately 60 days of enrollment.
3. Adverse Events will only be recorded for events which occur during or are believed to be associated with the stool sample collection procedure.
4. Subjects will have completed the study once the colonoscopy procedure has been completed or subject meets the withdrawal criteria in Section 9.3.

APPENDIX 3.0 – GUIDELINES FOR COLONOSCOPY

Bowel preparation procedures and post-enrollment colonoscopy will be performed following the established standard of practice at each clinical site. Results of the colonoscopy examination will be collected.

The quality of the colonoscopy bowel prep must be documented after washing/suctioning as follows:

- **Excellent -** minimal fecal residue and liquid, small volume of liquid easily aspirated, <5% of colonic surface covered;
- **Good -** small amounts of fecal residue, volume of clear liquid covering 5-25% of surface, easily aspirated;
- **Fair -** moderate amounts of fecal residue; stool limited examination but >90% could be examined; or
- **Poor -** large amounts of stool, <90% of surface could be examined.

Subjects with negative colonoscopy results and a poor bowel preparation will not be included in endpoint analyses.

Cecal intubation must be documented with photographic evidence and/or documentation of cecal intubation. Subjects with a negative colonoscopy will not be included in endpoint analyses if no documentation of cecal intubation is present.

For all subjects with histopathological findings of CRC (Category 1.0) or precursor lesions (Category 2.0), neither the quality of bowel preparation nor documentation of cecal intubation need to be present for the subject to be included in endpoint analyses.

APPENDIX 4.0 – GUIDELINES FOR PATHOLOGY REVIEW AND DOCUMENTATION

Sites must acquire and maintain reports of the histopathologic interpretation of endoscopic biopsies, polypectomy specimens, and excisional surgical pathology specimens, which should be available on all CRCs and precursor lesions (Category 2.0, inclusive).

Histopathology results will be collected on any excised or biopsied lesion. Lesion location within the colon (site), size (mm), will be recorded in the pathology report for all CRC's and precursor lesions. Table 1 provides codes for lesion locations.

Table 1: Index Lesion Location: Use reported location or insertion depth in cm.

P = Proximal Colon: cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, right colon NOS, [Insertion Depth >60 cm]

D = Distal Colon: descending colon, sigmoid colon, recto-sigmoid colon, left colon NOS, [Insertion Depth = 16 – 60 cm (inclusive)]

R = Rectal: Rectum, [Insertion Depth = 0-15 cm (inclusive)]

Lesions are also be categorized for study based on histopathologic diagnosis of the most clinically significant lesion (Index Lesion). Table 2 provides the listing of categories that will be used when assessing and determining final categorization of index lesions:

Table 2: Categorization of Subject for Study based on histopathologic diagnosis of the Index Lesion

Description	Category
Adenocarcinoma of colorectum, Stage I-IV	1.0
Advanced adenoma, including the following subcategories	2.0
Adenoma with HGD/CIS, any size	2.1
Adenoma with villous growth pattern (≥ 25%), any size	2.2
Adenoma ≥ 10 mm in size	2.3
Serrated lesion, ≥ 10 mm in size (SSA/SSP/HP)	2.4
Adenoma, non-advanced, >5.0, <10mm in size, 1 or 2 adenomas	3.0
Adenomas, non-advanced, < 10 mm in size, ≥ 3 adenomas	4.0
Adenoma, non-advanced, ≤ 5 mm in size, 1 or 2 adenomas	5.0
Negative: No Adenocarcinoma of the colorectum, No Adenomas or SSA/SSP or No HP ≥ 10 mm	6.0
Sub-categories Negative, no colorectal neoplasia upon histopathological review	6.1
No findings on colonoscopy, no biopsy(s) taken	6.2

Subjects will be categorized for this study into a single category (1.0 – 6.0) based on the most clinically significant lesion present (Index Lesion). For this study, the hierarchy of clinical significance in descending order of significance is 1.0 (highest clinical significance), then 2.1; 2.2; 2.3; 2.4; 3.0; 4.0; 5.0;

and 6.0 (lowest clinical significance). Table 3 provides the hierarchy of clinical significance regarding Index Lesions.

Table 3: Hierarchy of Clinical Significance of Index Lesion

Index Lesion Hierarchy (in Descending order)	Histopathology Category
Adenocarcinoma of the colorectum Stage I – IV (CRC, all stages)	1.0
Advanced adenoma with HGD/CIS, any size	2.1
Advanced adenoma with villous component (≥ 25%), any size	2.2
Advanced adenoma ≥ 10 mm in size, no HGD/CIS or villous component ≥ 25%	2.3
Serrated lesion ≥ 10 mm in size (includes Sessile serrated adenoma/sessile serrated polyp (SSA/SSP) and hyperplastic polyp (HP)	2.4
Adenoma, non-advanced, >5.0, <10mm	3.0
Adenoma, non-advanced, <10 mm, ≥ 3 adenomas	4.0
Adenoma, non- advanced, ≤ 5.0 mm	5.0
Hyperplastic polyp < 10 mm	6.0
Incidental findings (including malignancies other than colorectal adenocarcinoma)	N/A

A subject with CRC will be classified as CRC category (1.0), regardless of the presence of any other dysplastic or neoplastic lesions.

Subjects with pathologically confirmed non-advanced adenoma(s) but without CRC (1.0) or precursor lesions (Category 2.0 inclusive) will be classified into category 3.0, 4.0 or 5.0 based on the size and number of lesions. The largest of the non-advanced adenoma will be the index lesion for the case. In the case of subjects with both small (<10 mm) non-advanced adenomas and hyperplastic polyps < 10 mm, they will be classified based on the non-advanced adenomas only and will be classified into category 3.0, 4.0 or 5.0.

All subjects who do not have CRC (1.0), precursor lesions (Category 2.0 inclusive) or non- advanced adenomas (Categories 3.0-5.0) will be classified as negative (6.0). Category 6.0 includes subjects with any number of hyperplastic polyps <10mm and any non-neoplastic finding including evidence of active or chronic IBD, non-colorectal epithelial neoplasia (e.g. carcinoid, lipoma). In cases with hyperplastic polyp(s) < 10 mm, the largest hyperplastic polyp is the index lesion. In the absence of hyperplastic polyps, subjects in category 6.0 will either have “no index lesion” or will have had no pathology submitted.

In addition TNM stage and overall AJCC CRC Stage (I-IV) will be recorded for all CRC’s. Staging System to be used for Index Lesions categorized as Category (1) Tumor, Node, Metastasis (TNM) Definitions (Adapted from NCCN Guidelines and AJCC Cancer Staging System, 7th edition). Tis (CIS, intramucosal carcinoma) categorized as Category 2. Table 4 defines the TNM staging for cancers.

Table 4: TNM Staging for Cancers

	Primary Tumor (T) Description	**(Record as T: 1, 2, 3, 4, or X)**
TX	Primary tumor cannot be assessed	(Do not record subcategory a or b)
T1	Tumor invades submucosa	
T2	Tumor invades muscularis propria	
T3	Tumor invades through the muscularis propria into the pericolorectal tissues	
T4a	Tumor penetrates to the surface of the visceral peritoneum	
T4b	Tumor directly invades or is adherent to other organs or structures	
	Regional Lymph Nodes (N) Description	**(Record as N: 0, 1, 2, or X)**
NX	Regional lymph nodes cannot be assessed	(Do not record the subcategory a, b, or c)
N0	No regional lymph node metastasis	
N1	Metastasis in 1"3 regional lymph nodes	
N1a	Metastasis in 1 regional lymph node	
N1b	Metastasis in 2"3 regional lymph nodes	
N1c	Tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis	
N2	Metastasis in 4 or more regional lymph nodes	
N2a	Metastasis in 4"6 regional lymph nodes	
N2b	Metastasis in 7 or more regional lymph nodes	
	Distant Metastasis (M) Description	**(Record as M 0, 1, X)**
MX	Metastasis, distant, cannot be assessed	(Do not record the subcategory a or b)
M0	No distant metastasis	
M1	Distant metastasis	
M1a	Metastasis confined to one organ or site	
M1b	Metastasis in more than one organ/site or the peritoneum	

Colon Cancer Staging (TNM) AJCC 8th Edition

(Record as 1, 2, 3, 4, or X, do not record the sub-stage)

Stage	T	N	M
0	Tis	N0	M0
I	T1 - T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1 - T2	N1 / N1c	M0
	T1	N2a	M0
IIIB	T3 - T4a	N1 / N1c	M0
	T2 - T3	N2a	M0
	T1 - T2	N2b	M0
IIIC	T4a	N2a	M0
	T3 - T4a	N2b	M0
	T4b	N1 - N2	M0
IVA	any T	any N	M1
IVB	any T	any N	M1
IVC	any T	any N	M1

If stage cannot be assessed, record the AJCC Stage as “X”