Study Name: Colorectal Cancer and Pre-Cancerous Adenoma Non-Invasive Detection Test Study

NCT Number: 04739722

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Geneoscopy, Inc.

Confidential information has been redacted for public viewing.

GENEOSCOPY, INC.	**Title:** CRC-PREVENT Statistical Analysis Plan
	Document Number: CT-PLN-0002

Statistical Analysis Plan for the Clinical Validation of the mt-sRNA test: ColoRectal Cancer and Pre-Cancerous Adenoma Non-InvasiVE DetectioN Test Study

CRC-PREVENT

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Pro00045815 (Single-Site Protocol)

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1. Statistical Analysis Plan Approval Signatures

By signing below, I give my approval for the attached Statistical Analysis Plan for Geneoscopy's Clinical Validation Study entitled, "ColoRectal Cancer and Precancerous Adenoma Non-InvasiVE DetectionN Test Study" (CRC-PREVENT) Version 1 - Effective Date 12APR2021.

Approval Signatures

Credentials	**Name**	**Signature / Date**
Statistician	Carl Schaper, PhD	
Principal Investigator	Faith Holmes, MD	
Sponsor Lead	Erica K Barnell, PhD	

2. Abbreviations and Definitions

iFOBT / FIT	Fecal occult blood test / Fecal immunochemical test
IFU	Instructions for use
seRNA	Stool-derived eukaryotic RNA
CSR	Clinical Study Report
CRC	Colorectal cancer
AA	Advanced adenoma
OA	Other precancerous adenomas
FDA	Food and drug administration
SSA	Sessile serrated adenoma/polyp
TA	Tubular adenoma
VA	Villous adenoma
TVA	Tubulovillous adenoma
IRB	Institutional review board
Sen	Sensitivity
Sp	specificity
SOP	Standard operating procedure
ICF	Informed consent form
CSR	Clinical Study Report
SAP	Statistical Analysis Plan

3. Introduction

3.1 Preface

Colorectal cancer (CRC) is the third most common cancer in both men and women in the United States, the second deadliest cancer globally, and accounted for over 50,000 deaths in 2018 in the US alone. Disease onset is typically insidious, starting as a small polyp which can take several years to further accrue somatic mutations and develop into an invasive carcinoma. If detected early, CRC has a five-year survival rate of 92%. However, 63% of newly diagnosed patients have advanced disease, with an associated five-year survival rate as low as 14%. Late-stage diagnosis typically results from patient noncompliance with screening guidelines, indicating that these cancers could have been detected earlier by following standard of care recommendations. CRC screening compliance has remained stagnant over the past 20 years and is currently estimated to be approximately 60%. Historically, low compliance rates have been due to the inconvenience, unpleasantness, and perceived hazards of colonoscopies.

While colonoscopies are currently the gold-standard for colorectal cancer screening, the procedure does have associated risks to the patient. A colonoscopy procedure can result in an adverse event, which includes: pain (2.59%), hemorrhage (0.28%), perforation (0.05%). These complications can lead to hospitalization (1.17%), urgent care visit (2.34%), or in rare cases death (0.01%). An additional risk to the patient is a false negative result. It has been described that colonoscopies can miss 15-24% of all adenomas, 6-11% of advanced adenomas, and 1% of carcinomas.[1]

While many noninvasive tests have been developed to address compliance issues, none compare to the diagnostic accuracy of a colonoscopy. Currently, the most accurate noninvasive diagnostic (Cologuard, Exact Sciences) cites a CRC sensitivity of 92%, however, the advanced adenoma (AA) detection rate for this test is only 42%. Other noninvasive stool-based tests include the fecal occult blood test and the fecal immunochemical test (FIT), which use lateral flow for detection of blood in stool. These alternatives can be highly sensitive (79%) and specific (94%) for CRC, but have AA sensitivities of less than 30%. Accurate detection of precancerous adenomas would allow for preemptive excision of dysplastic tissue prior to carcinogenesis, thus reducing CRC incidence and the associated morbidity and mortality.

Geneoscopy has developed a multi-factor stool-RNA (mt-sRNA) assay that combines 8 stool-derived eukaryotic RNA (seRNA) biomarkers, patient demographic information (smoking status), and a fecal immunochemical test (FIT / iFOBT) to sensitively detect CRC, AA, and other precancerous adenomas. The mt-sRNA assay is undergoing analytical and clinical validation through a clinical trial entitled, CRC-PREVENT (NCT04739722). The results from CRC-PREVENT will be analyzed using the statistical analysis plan outlined in this document to assess safety and efficacy of the mt-sRNA assay.

3.2 Scope of the Analyses

The scope of the statistical analysis plan is to use the data from the CRC-PREVENT clinical trial to assess the safety and efficacy of the mt-sRNA assay produced by Geneoscopy. Specifically, results from the mt-sRNA test will be compared to results from a colonoscopy to ascertain sensitivity for positive lesions and specificity for negative lesions. Results from these analyses will be included in the clinical study report (CSR).

4. Study Objectives and Endpoints

4.1 Primary Study Objectives

The primary objective of this study is to determine safety and efficacy of Geneoscopy's mt-sRNA test for detection of colorectal neoplasias (i.e., colorectal cancer and advanced adenomas) using colonoscopy as the reference method. Lesion categories will be confirmed by histopathologic examination. Performance of the mt-sRNA test will be evaluated based on comparison of the mt-sRNA result with the histopathological category. The primary objectives will support clinical claims to be used in regulatory submissions. If acceptance criteria are met, findings pertaining to all primary and secondary objectives will be reported to the clinical community through academic presentations and in a peer-reviewed publication.

4.2 Study Endpoints

The primary objective of this study is to determine the ability of Geneoscopy's mt-sRNA test to detect colorectal neoplasias (i.e., colorectal cancer and advanced adenomas) when using colonoscopy as the reference method. Colonoscopy reports are assessed by verified pathologists to determine subject categories. The subject categories (see **Table 1**) are compared to the mt-sRNA test result (Positive or Negative) to assess primary and secondary outcomes.

Table 1. Methods for classifying study subjects into categories based on colonoscopy findings.

Category	Binary Category	Description
Colorectal Cancer (CRC)	Positive	Stage I-IV colorectal cancer, any size
Advanced Adenomas (AA)	Positive	High-grade dysplasia or ≥10 adenomas, any size Tubulovillous adenoma, any size Tubular adenoma, ≥10mm Traditional serrated adenoma, ≥10mm
Medium Risk Adenomas (MRA)	Negative	Hyperplastic polyp or SSL, ≥10mm 5-9 adenomas (TA + SSL), <10mm 3-4 adenomas (TA + SSL), <10mm
Low Risk Adenomas (LRA)	Negative	1-2 adenomas (TA + SSL), 5-9mm 1-2 adenomas (TA + SSL), <5mm
No Findings (NEG)	Negative	Hyperplastic polyps, <10mm Benign lesions, any size No lesions on colonoscopy

4.2.1 Primary Study Endpoints

The primary objective of this study will be assessed using four co-primary performance measures or study endpoints:

- mt-sRNA test sensitivity for subjects with colorectal cancer (CRC), which is the percentage of individuals with a diagnosis of colorectal cancer (**Table 1**) that were detected as positive by the mt-sRNA test.

- mt-sRNA test sensitivity for subjects with advanced adenomas (AA), which is the percentage of individuals with a diagnosis of advanced adenoma (**Table 1**) that were detected as positive by the mt-sRNA test.
- mt-sRNA test specificity for subjects with negative findings, which is the percentage of individuals with a diagnosis of benign polyps, or no findings on a colonoscopy (**Table 1**) that were detected as negative by the mt-sRNA test.

Criteria for success for these metrics requires the following:

- With regards to the mt-sRNA test sensitivity for subjects with CRC, the sensitivity of CRC must be greater than 90% and the lower bound of the 95% two-sided confidence interval must be greater than 80%.
- With regards to the mt-sRNA test sensitivity for subjects with AA, the sensitivity of AA must be greater than 45% and the lower bound of the 95% two-sided confidence interval must be greater than 40%.
- With regards to the mt-sRNA test specificity for subjects with no findings on a colonoscopy, the specificity must be greater than 80%.

4.2.2 Secondary Study Endpoints

The primary objective of this study will also be assessed using four co-secondary performance measures or study endpoints:

- mt-sRNA test sensitivity for subjects with high-grade dysplasia, which is the percentage of individuals with a diagnosis of carcinoma *in situ* or advanced adenoma with high grade dysplasia (**Table 1**) that were detected as positive by the mt-sRNA test.
- mt-sRNA test sensitivity for subjects with villous / tubulovillous adenomas, which is the percentage of individuals with a diagnosis of advanced adenoma with villous or tubulovillous growth pattern, any size (**Table 1**) that were detected as positive by the mt-sRNA test.
- mt-sRNA test sensitivity for subjects with sessile serrated adenomas / polyps, which is the percentage of individuals with a diagnosis of hyperplastic polyps or sessile serrated adenoma / polyp (SSA) ≧ 10mm any size (**Table 1**) that were detected as positive by the mt-sRNA test.

For each of these metrics, the two-sided 95% exact binomial confidence intervals will be provided.

5. Study Methods

5.1 General Study Design Plan

The CRC-PREVENT Clinical Validation study will assess the clinical sensitivity and clinical specificity of Geneoscopy's mt-sRNA test. Subject recruitment and activation for the CRC-PREVENT study will be managed by a validated clinical research organization (CRO). This entity will identify subjects online, ship a collection kit to the subject's residence, assist with stool sample collection, and navigate the subject to receive a standard of care colonoscopy. This optical colonoscopy procedure will serve as the reference method for the mt-sRNA test.

A subject will be determined as eligible if they complete an online screener and preliminarily meet eligibility criteria (see Section 5.2). Subjects will be determined as consented if they confirm eligibility criteria by a qualified study member from the CRO and sign the informed consent form (ICF) / medical data release forms. Subjects will be considered withdrawn from the study if they meet any of the criteria for withdrawal (see Section 5.2). Subjects will be considered completed, and eligible for primary and secondary objective analysis, if they have a valid mt-sRNA test score and a valid colonoscopy / colonoscopy category.

For a subject to have a valid mt-sRNA test score, the subject needs to complete the following:

- Obtain an mt-sRNA collection kit
- Complete the stool sample / FIT collection based on the instructions for use (IFU) prior to screening colonoscopy
- Ship the completed mt-sRNA test collection kit to Geneoscopy laboratories within 72 hours of stool sample production
- Have an mt-sRNA test score generated from the samples obtained in the mt-sRNA test collection kit

For a subject to have a valid colonoscopy / colonoscopy category, the following is required:

- Subject submits a stool sample prior to the colonoscopy
- Subject completes bowel preparation to ensure adequate visualization during the colonoscopy
- Subject completes a standard-of-care colonoscopy
- Histopathologic review and documentation of lesions removed during the colonoscopy is performed, as needed
- The subject's colonoscopy / histopathology reports must be sent to the CRO
- The subject categorization (see **Table 1**) must be completed based on reports submitted to the CRO

Stool samples eligible for the clinical study will be collected and assessed in accordance with mt-sRNA test's predefined standard operating procedures and the test's composite score will be generated using the validated mt-sRNA test Analysis Software. Lesions observed via colonoscopy will be confirmed by histopathology and classified based on findings listed in **Table 1**. mt-sRNA test scores and colonoscopy categories for all subjects will be blinded throughout data generation. Both datasets will be locked prior to unblinding.

After unblinding of the data, the two datasets will be merged to allow for assessment of primary and secondary objectives. Assessment will be performed by a third, unbiased statistician. A report will be generated based on findings.

5.2 Inclusion – Exclusion Criteria and General Study Population

Inclusion Criteria:

- Subject is male or female, >45 years of age
- Subject is able to understand the study procedures, and is able to provide consent to participate in the study and authorizes release of relevant protected health information through reviewing and consenting to a HIPAA medical release form
- Subject is able and willing to provide stool samples prior to a colonoscopy procedure.
- Subject is able and willing to undergo a colonoscopy after providing a stool sample

Exclusion Criteria:

- Subject had any precancerous findings on most recent colonoscopy. This does not include benign, and/or hyperplastic polyps of any size (Note: Tissue biopsies that result in no histopathology findings are acceptable)
- Subject has a history or diagnosis of colorectal cancer
- Subject has a history of aerodigestive tract cancer
- Subject has had a positive non-invasive screening diagnostic within the associated recommended intervals

  - Fecal occult blood test or fecal immunochemical test within the previous twelve (12) months
  - FIT-DNA test within the previous 36 months
- Subject has had a colonoscopy in the previous nine (9) years.
- Subject has had a prior colorectal resection for any reason other than sigmoid diverticular disease
- Indication for colonoscopy was due to overt rectal bleeding, e.g., hematochezia or melena, within the previous 30 days. (Blood on toilet paper, after wiping, does not constitute rectal bleeding)
- Subject has a diagnosis or personal history of any of the following high-risk conditions for colorectal cancer:
  - Inflammatory bowel disease (IBD) including chronic ulcerative colitis (CUC) and Crohn's disease
  - Familial adenomatous polyposis (also referred to as "FAP", including attenuated FAP)
  - Hereditary non-polyposis colorectal cancer syndrome (also referred to as "HNPCC" of "Lynch Syndrome")
  - Other hereditary cancer syndromes including but are not limited to Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner's Syndrome, Turcot's (or Crail's) Syndrome, Cowden's Syndrome, Juvenile Polyposis, Cronkhite-Canada Syndrome, Neurofibromatosis and Familial Hyperplastic Polyposis

Criteria for Withdrawal:

Subjects who enroll into the study but do not complete study requirements will be withdrawn from the analysis. Reasons for withdrawal include:

- Inadequate bowel preparation during colonoscopy
- Inadequate colonoscopy procedure
- Inadequate or incomplete medical records
- Inadequate or invalid specimen/sample
- Inadequate amount of stool provided with the sample
- Inadequate or invalid FIT
- Subject does not meet eligibility criteria upon review of medical records
- RNA quantification failure
- Other laboratory processing failure

Additionally, study subjects have the option to withdraw from the study at any point. If a subject is withdrawn from the study due to criteria listed above, or if the subject voluntarily withdrew from the study, data from their stool sample is not eligible for assessment during clinical validation.

5.3 Study Blinding

Mt-sRNA scores will be generated throughout the study duration by clinical trial study members. Final scores associated with each consented subject will be generated by a validated software and automatically populated into a Laboratory Information Management System (LIMS). Final batch records will be reviewed by personnel with delegated authority at Geneoscopy. Throughout mt-sRNA processing, all laboratory information, including mt-sRNA scores, will be blinded to individuals who have access to colonoscopy reports / results.

Once mt-sRNA test processing has been completed, a final dataset will be generated for all consented subjects. At this time, access to the LIMS system will be restricted to view access only for study personnel. The final dataset will be locked and undergo document control processes, per standard operating procedures. Once the

final dataset has been approved, data lock of the laboratory component (i.e., mt-sRNA test results) will be considered complete.

Colonoscopy / histopathology results will be obtained by Geneoscopy's selected CRO, throughout the study duration, per predefined standard operating procedures by clinical trial study members. Reports will be uploaded to a validated colonoscopy database along with other relevant subject information. Reports will be reviewed by board certified pathologists. Final report reviews will be reviewed by individuals with delegated authority at the CRO. Throughout report collection, all colonoscopy information will be blinded to individuals who have access to mt-sRNA test reports / results.

Once colonoscopy / histopathology report collection and analysis has been completed, a final dataset will be generated for all consented subjects. At this time, access to the validated colonoscopy database will be restricted. The final dataset will be locked by the validated CRO. Once the final dataset has been approved, data lock of the colonoscopy component will be considered complete.

After data lock of both the colonoscopy component and the laboratory component, unblinding will occur. At that time, the two datasets will be merged based on unique subject identifiers. The merged dataframe will be provided to an unbiased statistical analysis team who will use the final dataset to assess primary and secondary objectives. Results from this analysis will be provided to study members.

5.4 Study Assessments

A variety of categorical data analysis methods will be used to analyze the results from the mt-sRNA test and the reference colonoscopy results. Binomial proportions together with exact 95% confidence intervals will be used to summarize sensitivity and specificity for various categories. Fisher exact tests will be used for determining whether there are significant differences in assay performance between various subgroups, e.g., are there differences in sensitivity for CRC between females and males. If there are statistically significant (α=0.05) differences observed, logistic regression will be used to perform any modeling to better understand differences. All data will be summarized into tables based on the outlines provided below. An executed analysis will be provided as a report based on the findings.

6. Sample Size

The overall sample size for the study is driven by the need to obtain enough CRC cases so that the lower 95% exact confidence limit for the sensitivity is at least 80%. **Table 3** shows the smallest number of CRC cases that would meet this condition for each of 0 - 5 False Negatives.

Table 3. CRC cases required to meet primary objective criteria

Number of CRC Cases	# False Negative	Observed Sensitivity	Lower 95% CL	Upper 95% CL
17	0	100.0%	80.5%	100.0%
26	1	96.2%	80.4%	99.9%

34	2	94.1%	80.3%	99.3%
41	3	92.7%	80.1%	98.5%
48	4	91.7%	80.0%	97.7%
55	5	90.9%	80.0%	97.0%

A sample size of 48 would allow up to 4 false negatives corresponding to an observed sensitivity of 91.7%. Assuming a prevalence of 0.6% in the intended use population, a starting sample size of 8,500 will have a 68% chance of yielding at least 48 CRC cases. Given the low prevalence of CRC cases, a small difference between the actual prevalence and the postulated prevalence could lead to too few CRC cases.

The number of CRC cases will be tracked by non-clinical study members at Geneoscopy's selected CRO during pathology review of colonoscopy reports. Enrollment will be arrested when a sufficient number of confirmed cases of CRC have been identified among the cohort to achieve study endpoints.

7. General Analysis Considerations

7.1 Timing of Analyses

The CRC-PREVENT clinical trial was launched in June of 2021. Subject enrollment continued through June of 2022. During that time frame, approximately 14,000 subjects were consented onto the clinical trial. All subjects received the same opportunity to complete clinical trial requirements. This included:

- consenting to clinical trial informed consent
- providing medical data release forms to obtain medical records
- providing a viable stool sample for mt-sRNA test analysis
- completing a screening colonoscopy
- submitting medical records for colonoscopy review

7.2 Analysis Population

If a subject was unable to complete the following requirements and/or the subject was withdrawn for any reason (see Section 5.2), then the subject will not be eligible for primary or secondary analysis. For each individual, the mt-sRNA test analysis eligibility and the colonoscopy eligibility will be determined separately. These determinations will be included in final locked datasets prior to unblinding. If a subject is eligible in both datasets, they will be eligible for primary and secondary analysis. A subset of ineligible samples will be analyzed as part of tertiary study endpoints.

7.2.2 Per Protocol Population

Samples that were associated with major protocol deviations to the Clinical Trial were identified. All samples that are associated with a major protocol deviations will be labeled in the final locked dataset. Primary and Secondary analysis will be performed for all samples as well as for samples with a major protocol deviation. This analysis will be reported as a tertiary study endpoint.

7.2.3 Safety Population

All subjects who are enrolled into the clinical trial are provided information related to safety endpoints and are educated on reporting any observed or perceived adverse device effects which may also be referred to as adverse events during informed consent. Subjects who experience an adverse event during the course of study execution are considered still eligible as part of the study population unless the event precludes them from properly completed the required elements for study completion.

7.3 Covariates and Subgroups

All covariates and subgroup analyses will be outlined as primary, secondary, and tertiary analyses.

7.4 Missing Data

Given the study design, if material data is missing for a given subject (e.g., stool sample or colonoscopy results), then the subject must be withdrawn from the study and will not be eligible for primary and secondary analysis. If there are components of the test record that are missing for individuals, these will be considered major deviations from the clinical study protocol and will be analyzed using a subgroup analysis as a tertiary study endpoint.

7.5 Data Monitoring

As per the CRC-PREVENT Clinical Trial Protocol, data collected during trial execution will require monitoring. Monitoring visits will occur during clinical trial execution and at clinical trial termination, as per CT-SOP-0006 Clinical Trial Monitoring and the Clinical Monitoring Plan CT-PLN-0001. During monitoring visits, site-specific documentation as well as subject-specific documentation will be assessed. Site-specific documentation includes the Trial Master File (TMF) as well as Investigator Site Files (ISF) for each site associated with the clinical trial. Subject-specific documentation will be collected and stored in secure centralized databases at associated clinical trial sites. Geneoscopy will maintain the database associated with mt-sRNA test results and Geneoscopy's selected CRO will maintain the database associated with the colonoscopy results. Data within these secure databases will be obtained from five sources:

- **Eligibility Survey:** The responses to the eligibility survey will be obtained from the CRO using a secure API.
- **Tracking Information**: Sample tracking information will be collected using a courier's online portal.
- **Sample Information**: Data about the sample and collection kit will be obtained. This will be collected by study staff who are accessioning the sample at Geneoscopy's Laboratories.
- **Sample Processing Information**: During processing at Geneoscopy's testing site, data that is generated will be obtained and stored using a secure central database.
- **Clinical Data**: Subject medical reports will be obtained from the subject's physicians.

All data stored in the secure centralized databases will be subject to CT-REC-0003 Data Management

Plan. Findings observed during monitoring activities that impact the statistical analysis can be used to revise this document.

7.6 Repeat / Multiple Testing

For a subject to be considered eligible for inclusion in clinical trial endpoints, the subject must provide a valid stool sample using Geneoscopy's mt-sRNA test Collection Kit. There are some instances whereby a subject might have received multiple Collection Kits. If a subject submits multiple stool samples via multiple Collection Kits, all kits will be evaluated for viability. If multiple kits are viable, based on protocol requirements, then the most recent collection kit will be used for primary and secondary analyses. Results across multiple collection kits can be compared as tertiary study endpoints.

Additionally, each Collection Kit received by the laboratory contains enough stool to assess the mt-sRNA test up to two times. Replicate #1 is referred to as the "RNA" replicate and Replicate #2 is referred to as the "STG" replicate. Per standard operating procedures, the RNA replicate is analyzed first. If the RNA replicate is not able to be analyzed or provides an invalid mt-sRNA test result, then the STG replicate is extracted and analyzed. If the STG replicate is not able to be analyzed or provides an invalid mt-sRNA test result, then the sample is considered invalid. In the final locked laboratory dataframe, for each eligible subject, the viable collection kit and the viable replicate that will be used for primary and secondary study endpoints will be defined prior to unblinding. Results across multiple replicates can be compared as tertiary study endpoints.

7.7 Interim Analysis

The CRC-PREVENT study will not utilize an interim analysis to assess the primary or secondary objectives of the clinical trial.

8. Summary of Study Data

8.1 Subject Disposition

Screening survey: This may occur within 1 day or over a number of days.

- Obtain subject information
- Determine initial eligibility
- Obtain subject demographics

Initial contact from the call center: This may occur within 1 day or over a number of days.

- Confirm eligibility
- Sign informed consent form
- Sign medical data release form(s)
- Dispense the mt-sRNA test Collection Kit
- Schedule colonoscopy and/or obtain procedure information
- Enter all subject data into the data portal

Stool collection: Complete prior to the colonoscopy preparation / procedure

- Subject is instructed to complete the collection prior to colonoscopy preparation and procedure

- Subject collects stool sample and FIT/iFOBT swab
- Subject attests to sample collection
- Subject will indicate if a repeat sample collection is necessary and study member will initiate a subsequent collection kit to be sent to the subject's residence

Stool assessment**:** Completed upon receipt of the stool sample in Geneoscopy's laboratory

- Laboratory technologists will receive collection kit
- Technologists will accession stool samples and FIT/iFOBT
- FIT/iFOBT will be analyzed
- Sample will be processed using the molecular component of the mt-sRNA test
- mt-sRNA test score is generate for the subject
- Study members determine if a repeat stool sample collection is necessary and arranges for subsequent collection kit to be sent to the subject's residence

Bowel preparation**:** Must be initiated after stool collection

- Subject performs bowel preparation according to the instructions provided by the facility where the procedure is being performed

Colonoscopy procedure**:** Must be initiated after stool collection

- Subject undergoes standard of care screening colonoscopy
- Endoscopist will send any lesions removed during the colonoscopy to histopathology

Record retrieval**:** Initiated approximately 3 weeks after colonoscopy procedure

- A verified CRO will call the endoscopy center and provide medical data release forms to obtain colonoscopy and histopathology results
- Reports from the colonoscopy and histopathology will be sent to the verified CRO
- Data will be entered in the secure portal

Pathology review**:** Can be completed at any time

- Study member determines if a repeat colonoscopy is needed or if the subject is ineligible based on reports
- Two pathologists independently review the reports
- Algorithm pairs the pathology classifications and determines if a tie-break review, or subsequent review, is necessary
- Final classifications for each study member will be determined

Study termination**:** Date of obtaining the last report required to meet study criteria

- Study members will determine if a subject is eligible based on laboratory results
- Study members will determine if a subject is eligible based on colonoscopy results
- Datalock will occur for both the laboratory component and the colonoscopy component
- Study members will become unblinded to results
- Data analysis will commence

8.2 Derived Variables

There are no known derived variables that will be used as part of the statistical analysis of primary and

secondary endpoints.

8.3 Protocol Deviations

Planned protocol deviations will be employed prior to execution of this statistical analysis plan. Unplanned protocol deviations will be recorded using Geneoscopy's standard deviation protocols. All deviations will be reported in the clinical trial study report.

8.4 Demographic and Baseline Medical Conditions

Due to the nature of this study, any individual over the age of 45 who is at average-risk for developing colorectal cancer is eligible for primary and secondary analysis. Any medical condition that increases risk for individuals development of CRC (see eligibility criteria) would make a person ineligible. All individuals are screened for eligibility criteria prior to enrollment into the clinical trial.

8.5 Concurrent Illnesses and Medical Conditions

All subjects with concurrent illness and medical conditions, so long as they do not impact eligibility criteria, are permissible for the clinical trial.

9. Efficacy Analyses

9.1 Primary Efficacy Analysis for Primary Study Endpoints

A variety of categorical data analysis methods will be used to analyze the results from the mt-sRNA test and the reference colonoscopy results. Binomial proportions together with exact 95% confidence intervals will be used to summarize sensitivity and specificity for various categories. Fisher exact tests will be used for determining whether there are significant differences in assay performance between various subgroups, e.g., are there differences in sensitivity for CRC between females and males. If there are statistically significant (α=0.05) differences observed, logistic regression will be used to perform any modeling to better understand differences.

Table 5. Data summaries will be generated for demographic and risk characteristics.

Demographics	**Total % (n)**	**CRC (1) % (n)**	**AA (2) % (n)**	**OA (3) % (n)**	**OA (4) % (n)**	**NEG (5) % (n)**
Age 45-55 55-65 65-75 75+	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)

Smoking Never smoked Previous smoker Currently smoke	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Sex Female Male No Answer	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Family History of CRC No Yes	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Racial Background American Indian / Alaska Native African American / Black Asian White Native Hawaiian / Pacific Islander Other Prefer not to answer	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Ethnic Background Hispanic Non-Hispanic Other Prefer not to answer	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Average Income $200,000 or More $150,000-$199,999 $100,000-$149,999 $75,000-$99,999 $50,000-$74,999 $30,000-$49,999 Under $29,999 Prefer not to answer	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)

Insurance No Insurance Private Insurance Public Insurance (Medicaid) Public Insurance (Medicare Advantage) Public Insurance (Medicare) Self-Insured	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Geographic Rural Urban / Suburban	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)

Table 6. Data summaries for subject categories will be generated for sensitivity and specificity

Category	Label	True Outcome	Colonoscopy Findings	% (N)
1	Colorectal Cancer	Positive	Stage I-IV colorectal cancer, any size	x (xx)
2	Advanced adenoma	Positive	High-grade dysplasia or ≥10 adenomas, any size Tubulovillous adenoma, any size Tubular adenoma, ≥10mm Traditional serrated adenoma, any size	x (xx)
3	Other adenoma	Negative	Hyperplastic polyp or SSL, ≥10mm 5-9 adenomas (TA + SSL), <10mm 3-4 adenomas (TA + SSL), <10mm	x (xx)
4	Other adenoma	Negative	1-2 adenomas (TA + SSL), 5-9mm 1-2 adenomas (TA + SSL), <5mm	x (xx)
5	Negative	Negative	Hyperplastic polyps, <10mm Benign lesions, any size No lesions on colonoscopy	x (xx)

Table 7. Data summaries for all subjects will be summarized in a frequency table:

Mt-sRNA Test Result	Histopathological Lesion Category Grouping					
	CRC (1) % (n)	**AA (2) % (n)**	**OA (3) % (n)**	**OA (4) % (n)**	**NEG (5) % (n)**	Total
Positive	a	b	c	d	e	a+b+c+d+e
Negative	f	g	h	i	j	f+g+h+i+j
Total	a+f	b+g	c+h	d+i	e+j	N

The primary endpoint metrics will be calculated as:

- Mt-sRNA test sensitivity for subjects with CRC = a / (a+f)
- Mt-sRNA test sensitivity for subjects with AA = b / (b+g)
- Mt-sRNA test specificity for subjects with negative findings = j / (e+j)

For each of these metrics, the two-sided 95% exact binomial confidence intervals will be provided, and the lower 95% confidence limits will be compared to the acceptance criterion. An additional sensitivity calculation will be presented for subjects with Other Adenomas.

- Mt-sRNA test sensitivity for subjects with OA(3) = c/(c+h)
- Mt-sRNA test sensitivity for subjects with OA(4) = d/(d+i)

The two-sided 95% confidence interval will be calculated for this metric although there is no specific acceptance criterion against which it will be compared.

9.2 Secondary Efficacy Analysis for Primary Study Endpoints

Using the data from Table 5 - Table 7, above, the positive predictive value (PPV) for various clinical outcomes and the negative predictive value (NPV) will be calculated as follows, assuming underlying prevalences of the various disease categories that match those observed in the clinical study:

- PPV (CRC) = Probability of having CRC given a positive Mt-sRNA Test Result = a / (a+b+c+d+e)
- PPV (AA) = Probability of having AA given a positive Mt-sRNA Test Result = b / (a+b+c+d+e)
- NPV = Probability of having negative findings on Colonoscopy given a negative Mt-sRNA Test Result = j / (f+g+h+i+j)

The above straightforward calculations are possible under the assumption that underlying disease prevalences observed in the clinical validation study match real-world prevalences. In the event that observed prevalences

differ from expected prevalences, PPV and NPV calculations will also be performed for alternative assumed prevalences. In that case, PPV and NPV will be calculated by Bayes theorem using the alternative assumed prevalences together with the study estimates of the sensitivity and specificity components.

An alternative characterization of diagnostic performance can be made via diagnostic likelihood ratios (DLRs), which indicate how much greater the odds are that a subject has or does not have the condition once the test result is available.

Three DLRs will be calculated:

- positive DLR (CC) = P(Test positive | CRC) / P(test positive | No CRC) = a / (b+c+d+e)
- positive DLR (AA or CC) = P(Test positive | AA or CC) / P(test positive | No AA nor CC) = (a+b) / (c+d+e)
- negative DLR (Negative finding) = P(Test negative | Negative findings) / P(test negative|AA or CC or OA) = j / (f+g+h+i)

9.3 Analysis for Secondary Study Endpoints

For Secondary Study Endpoints, a variety of categorical data analysis methods will be used to analyze the results from the Mt-sRNA test and the reference colonoscopy results. Binomial proportions together with exact 95% confidence intervals will be used to summarize sensitivity and specificity for various categories. Fisher exact tests will be used for determining whether there are significant differences in assay performance between various subgroups, e.g., are there differences in sensitivity for CRC between females and males. If there are statistically significant (α=0.05) differences observed, logistic regression will be used to perform any modeling to better understand differences.

Table 8. Performance Characteristics by Cancer Stage

Subtype	**CRC Stage**	**CRC Sensitivity**	**95% confidence Interval**
Category 1.0	Stage 0	n = X n/N (%)	95% CI
	Stage I	n = X n/N (%)	95% CI
	Stage II	n = X n/N (%)	95% CI
	Stage III	n = X n/N (%)	95% CI
	Stage IV	n = X n/N (%)	95% CI

p-value		

Table 9. Performance Characteristics by Lesion / Advanced Adenoma Subtype

Subtype	**Lesion Type**	**Sensitivity**	**95% confidence interval**
Category 2.1	high-grade dysplasia / carcinoma in-situ	n = X n/N (%)	95% CI
Category 2.2	villous / tubulovillous	n = X n/N (%)	95% CI
Subset of category 2.2 + Category 3.1	sessile serrated adenomas / polyps	n = X n/N (%)	95% CI
p-value			

The secondary endpoint metrics will be calculated as:

- mt-sRNA test sensitivity for subjects parsed by cancer staging (Stage 0 - Stage IV) based on relevant guidelines.
- mt-sRNA test sensitivity for subjects with high-grade dysplasia / carcinoma in-situ
- mt-sRNA test sensitivity for subjects with villous / tubulovillous adenomas
- mt-sRNA test sensitivity for subjects with sessile serrated adenomas / polyps

If any differences are noted between any categories in the subgroup analyses, logistic regression will be used for further exploration to understand the cause of the differences.

10. Safety Analyses

With respect to safety, due to the design of the study and the nature of the stool collection process, adverse device effects (ADEs) caused by or related to the stool collection procedure are not anticipated. In the unlikely event that an ADE occurs, it will be evaluated by the PI and reported to the IRB and/or the FDA in accordance with CT-SOP-0004 Clinical Trial Adverse Effects. Any adverse device effect that is reported by a subject will be evaluated using CT-FRM-0007 to determine if the event was serious and/or unanticipated. All adverse device effects will be included in the final clinical trial report. For each reported adverse device effect, a general description, outcome, and reporting requirements will be included.

11. References

1. Inadomi, J. M. Screening for Colorectal Neoplasia. *New England Journal of Medicine* vol. 376 149–156 (2017).