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

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GENEOSCOPY, INC.	**Title:** CRC-PREVENT Clinical Validation Protocol
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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)
Version 1

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Table of Contents

0. Abbreviations

iFOBT	fecal occult blood test
FIT	fecal immunochemical test
IFU	instructions for use
seRNA	stool-derived eukaryotic RNA
ddPCR	droplet digital polymerase chain reaction
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

1. Background and Rationale

1.1 Study Synopsis

Sponsor	Geneoscopy Inc. 2220 Welsch Industrial Court St. Louis, MO 63146
Primary Objectives	The primary objective of the ColoRectal Cancer and Pre-Cancerous Adenoma Non-InvasiVE DetectioN Test Study study (CRC-PREVENT) is to determine sensitivity for colorectal cancer, advanced adenomas, and specificity for other precancerous adenomas, hyperplastic polyps, other lesions, and no findings on a colonoscopy for Geneoscopy's multi-target stool RNA assay (mt-sRNA) using colonoscopy as the reference methods. Lesions will be confirmed by histopathologic examination.
Sites	Recruitment for this screening study will utilize a decentralized model. It is estimated that participants recruited for this study will be derived from all 48 contiguous United States and will visit over 6,000 different clinical sites for endoscopy procedures.
Population	Participants over the age of 45, who are at average-risk of developing colorectal neoplasms and are considered enrolled after understanding and consenting to the informed consent form (ICF).
Investigational Device	Geneoscopy's mt-sRNA Test
Primary endpoint	The co-primary endpoints for this study are sensitivity and specificity of the mt-sRNA test for colorectal cancer, advanced adenomas, other precancerous adenomas,

	hyperplastic polyps, and no findings on a colonoscopy, with comparison to the colonoscopy result and histopathologic diagnosis of all lesions discovered during colonoscopy.
Study Procedures	This study will be a prospective analysis conducted by Geneoscopy Inc. to evaluate the multi-target stool RNA test (mt-sRNA test) for colorectal neoplasia screening. Patients who are eligible for a colonoscopy (i.e., asymptomatic, average risk individuals over the age of 45 who are not up-to-date with screening guidelines) will be enrolled in the study. Stool sample collection kits will be sent to individuals who enroll in the study. The collection kit provides information on how to collect a stool swab using a fecal immunochemical test (FIT / iFOBT) and how to collect a stool sample. After a stool sample is produced, patients will swab the stool sample using the iFOBT Sampling Bottle according to instructions for use (IFU) and will return the stool sample via mail courier to Geneoscopy's Laboratories. After samples are sent to the labs, participants will be directed to receive a colonoscopy. If a lesion is removed during the colonoscopy, the participant's tissue sample will be sent for histopathology review. Histopathology is the review of the tissue by a pathologist to determine if the tissue is benign, premalignant, or malignant. The colonoscopy report, physician reports, and the histopathology report will be provided to Geneoscopy. Stool samples returned to Geneoscopy's Laboratories by the participant will be subjected to the mt-sRNA test system, which includes targeted nucleic acid enrichment and biomarkers quantification via droplet digital polymerase chain reaction (ddPCR). The iFOBT Sampling Bottle returned in the Collection Kit will also be read by laboratory technologists at Geneoscopy Inc. This study will be used for validation of the investigational device.
Study Duration	Once a participant is enrolled in the study, the subject will produce a stool sample. Participants will be requested to submit the within 120 days of colonoscopy completion. Subjects will not be evaluated after the colonoscopy procedure, however all reports associated with findings from the colonoscopy report will be collected. These reports will be centrally reviewed. Subjects may be contacted by the investigation site for up to five (5) years following completion of the study to gather additional information.

1.2 Background Information

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.[1,2] 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.[3–5] 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%.[1,6,7] Late-stage diagnosis typically results from patient noncompliance with screening guidelines, indicating that these cancers could have been detected earlier by following standard protocols. CRC screening compliance has remained stagnant over the past 20 years and is currently estimated to be approximately 60%,[1,8] which is well below the National Colorectal Cancer Roundtable's goal of 80%.[8] In addition, nearly a quarter of all adults in at-risk populations have never before been screened.[7] Historically, low compliance rates have been due to the inconvenience, unpleasantness, and perceived hazards of colonoscopies. In an open-ended survey of 660 patients regarding the most important

barrier to CRC screening, three of the top four responses cited were directly related to the invasive nature of a colonoscopy: "afraid/fear," "prep unpleasant," and/or "anticipated pain".[9] Therefore, it is likely that a noninvasive alternative screening method would significantly increase the number of patients opting for CRC screening.

While colonoscopies are currently the gold-standard for colorectal cancer screening, the procedure does have associated risk 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%).[10] 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.[11–13] Miss rates are associated with withdrawal time, quality of bowel preparation, colonoscopy techniques, and polyp characteristics.[14]

While many noninvasive tests have been developed to address compliance issues, none compare to the diagnostic accuracy of a colonoscopy.[15] Currently, the most accurate noninvasive diagnostic (Cologuard, Exact Sciences) cites a CRC sensitivity of 92%.[16] However, the advanced adenoma (AA) detection rate for this test is only 42%.[16] 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%.[17] 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.[4,18]

Detection of precancerous lesions, including advanced and non-advanced adenomas, is essential for cancer prevention. Precancerous adenomas are categorized by both size and pathology. Advanced adenomas and non-advanced adenomas include the following findings on a colonoscopy:

- ≥10 adenomas of any size, shape, or pathology classification
- adenoma with high-grade dysplasia
- adenoma with carcinoma *in-situ*
- adenoma with villous architecture
- adenoma with tubulovillous architecture
- tubular adenoma ≧ 10mm
- 5-10 tubular adenomas
- Sessile serrated adenoma / polyp ≧ 10mm
- 5-10 tubular or sessile serrated adenomas / polyp
- 1-2 tubular or sessile serrated adenoma / polyp between 5-10mm
- >20 hyperplastic polyps
- Hyperplastic polyp ≧ 10mm
- 3-4 tubular or sessile serrated adenoma / polyp <10mm
- 1-2 tubular or sessile serrated adenoma / polyp ≦5mm

Malignant transformation rates[19] and recommended screening intervals[20] for each of the above lesions vary. Lesions with the most aggressive natural history include those with high-grade dysplasia or carcinoma *in-*

situ with an annual malignant transformation rate of 50%. Other lesions with high acuity include those with tubulovillous or villous architecture with an annual malignant transformation rate of 30-50% and sessile serrated adenomas / polyps (2-3 crypts) with an annual malignant transformation rate of approximately 10%. The malignant transformation rate for other adenomas is between 0.25-5%. For patients with fewer than 20 small hyperplastic polyps or patients with no findings on a colonoscopy, the risk of development of a malignant lesion is unlikely.

Given the range of genomic variants that can lead to malignant transformation of healthy colonic tissue, genomic assays that target a small number of canonical variants are not sufficiently sensitive for precancerous lesions.[4] However, colorectal cancer, advanced adenomas, and other precancerous adenomas can be detected by evaluating a panel of RNA biomarkers that encompass the broader effects of multiple pathological variants.[21,22] While the analysis of human RNA biomarkers in stool samples has been extremely challenging due to extensive RNA degradation and a high bacterial transcript burden, Geneoscopy has developed a method to reliably extract and evaluate stool-derived eukaryotic RNA (seRNA) biomarkers.[23,24] Using this extraction method, Geneoscopy has developed a multi-factor mt-sRNA test that combines 8 stool-derived eukaryotic RNA (seRNA) biomarkers, patient demographic information (smoking status), and a fecal immunochemical test (FIT / iFOBT) to sensitively detect colorectal cancer, advanced adenomas, and other precancerous adenomas.

Biomarker discovery for the mt-sRNA test was completed using a 264-patient cohort.[25] In this study, 639 transcripts associated with malignancy were assessed for association with advanced adenomas and colorectal cancer. Using a 154-patient training set and a 110-patient testing set, a final cohort of seRNA biomarkers were identified for the molecular component of the mt-sRNA test. A subsequent feasibility study was conducted using a multi-center, prospective study design.[26] For this clinical trial, 1,305 participants were recruited to participate using an online enrollment strategy. Participants received a collection kit in the mail, deposited a stool sample, and shipped the sample back to Geneoscopy's centralized lab. After processing the sample using the mt-sRNA test, participants were directed to receive a colonoscopy at their local endoscopy center. mt-sRNA test results were compared to the colonoscopy results to determine mt-sRNA test accuracy. The hold out testing set (n = 366 participants) demonstrated a 100% sensitivity for colorectal cancer (n = 2), a 60% sensitivity for advanced adenomas (n = 50), a 25% sensitivity for other precancerous adenomas (n = 139), 80% sensitivity for hyperplastic polyps (n = 74), and 85% sensitivity for no findings on a colonoscopy (n = 101). To improve confidence surrounding the cancer sensitivity, 20 samples were obtained retrospectively from patients with known diagnosis of colorectal cancer prior to surgical resection or therapy. The mt-sRNA test identified 19 of these 20 patients as positive (95% sensitivity).

The CRC-PREVENT clinical validation study described here will assess the clinical sensitivity and clinical specificity of the mt-sRNA test. A clinical research organization (CRO) will be involved with patient recruitment. This entity will direct patients through providing a stool sample to Geneoscopy and conducting a standard of care colonoscopy. This optical colonoscopy procedure will serve as the reference method for the mt-sRNA test. Lesions observed via colonoscopy will be confirmed by histopathology and classified based on findings listed in **Table 1**. 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 Analysis Software. The results from the colonoscopy and

histopathology will be blinded to laboratory technologists at Geneoscopy. To assess the accuracy profile, colonoscopy results will be stored by a second clinical research organization (CRO). Ultimately, Geneoscopy study members will supply the CRO with mt-sRNA test results. Study members at the CRO will link mt-sRNA test results to colonoscopy results to assess the primary endpoints of this study.

Table 1. Histopathological category definitions

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

2. Objectives

2.1 Primary and Secondary Objectives

The primary objective of this study is to determine the sensitivity and specificity of Geneoscopy's mt-sRNA test for 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 test result with the histopathological category (**Table 1**). 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.

Four co-primary performance measures are specified as:

- 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.

There will also be three co-secondary performance measures:

- 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. 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%.

These will be considered as a joint hypothesis as follows:

H_0: $CRC_{Sen} \leq 0.90$ or $AA_{Sen} \leq 0.45$ or $Sp \leq 0.80$
H_1: $CRC_{Sen} > 0.65$ and $AA_{Sen} > 0.45$ and $Sp > 0.80$

where CRC_{Sen} is the mt-sRNA test sensitivity for subjects with CRC, AA_{Sen} is the mt-sRNA test sensitivity for subjects with AA and Sp is the specificity for no findings on a colonoscopy. The associated p-values for two-sided 0.05 exact binomial tests will also be provided.

3. Subject Selection

3.1 Intended use population

The intended use population for the mt-sRNA test will include men and women who are 45 or older and are of average-risk for development of colorectal cancer and are asymptomatic of gastrointestinal symptoms that would warrant a diagnostic colonoscopy. To increase the point prevalence of CRC in subjects recruited for the study, subject enrollment will include the following:

- Inclusion of individuals with a family history of colorectal cancer
- Enrichment of the 65 and older age range, provided it does not exceed 60% of the total study population

3.2 Eligibility Criteria

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

3.3 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:

- Withdrew consent to participate in study
- Inadequate prep
- Inadequate procedure
- Inadequate records
- Inadequate specimen/sample
- Inadequate stool sample
- Inadequate FIT
- Review of eligibility criteria
- Process validation
- Quantification failure
- Other Processing failure

3.4 Inclusion of Women and Minorities

- Both men and women and members of all races and ethnic groups are eligible for this trial

3.5 Sample Size Requirements

The overall sample size 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 2 shows the smallest number of CRC cases that would meet this condition for each of 0 - 5 False Negatives.

Table 2. Cases 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 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 unblinded study members at the CRO to determine when to stop enrollment. Enrollment will be arrested when a sufficient number of confirmed cases of colorectal cancer have been identified among the cohort to achieve study endpoints. To assess cessation of enrollment, an initial 8,500 participants will be recruited into the study. After this initial enrollment the number of confirmed CRC cases will be assessed against the required number of CRC cases by unblinded study members. If the total number of required cases have not been met after recruitment of 8,500 patients, additional participants will be recruited in 1,000-patient intervals. Enrollment will continue until all endpoints are fully powered.

3.5 Primary Statistical Analysis Plan

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 3. Data summaries will be generated for demographic and risk characteristics.

Demographics	**Total % (n)**	**CRC % (n)**	**AA % (n)**	**OA % (n)**	**Specificity% (n)**
Age 45-55 55-65 65-75 75+	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)
Smoking	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)

Never smoked Previous smoker Currently smoke					
Sex Female Male No Answer	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)
Ethnic 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)
Race Hispanic Non-Hispanic Other Prefer not to answer					
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)
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)
Geographic Rural Urban / Suburban	x (xx)	x (xx)	x (xx)	x (xx)	x (xx)

Table 4. Data summaries for all subjects will be summarized in a frequency table:

Mt-sRNA Test Result	Histopathological Lesion Category Grouping				
	CRC	AA	OA	Negative	Total
Positive	a	b	c	d	a+b+c+d
Negative	e	f	g	h	e+f+g+h
Total	a+e	b+f	c+g	d+h	N

The primary endpoint metrics will be calculated as:

- mt-sRNA test sensitivity for subjects with CRC = a / (a+e)
- mt-sRNA test sensitivity for subjects with AA = b / (b+f)
- mt-sRNA test specificity for subjects with negative findings = h / (d+f)

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 = c/(c+g)

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.

3.6 Additional Metrics of mt-sRNA test Performance

Using the data from the above tables, 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)
- PPV (AA) = Probability of having AA given a positive mt-sRNA test result = b / (a+b+c+d)
- NPV = Probability of having negative findings on Colonoscopy given a negative mt-sRNA test Result = h / (e+f+g+h)

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)
- positive DLR (AA or CC) = P(Test positive | AA or CC) / P(test positive | No AA nor CC) = (a+b) / (c+d)

- negative DLR (Negative finding) = P(Test negative | Negative findings) / P(test negative|AA or CC or OA) = (h) / (e+f+g)

The calculation of the four metrics will be repeated for subgroups based on 10-year age ranges, gender and ethnicity. Results will be reported in tables as follows. For the given colonoscopy result, i.e., within each column, a Fisher exact test will be used to test whether the mt-sRNA test gives different proportions of positive / negative test results across the categories specified by the different rows.

Table 6. Performance Characteristics by Age Group

Age Range	Sensitivity			Specificity
	CRC	AA	OA	
45-55	n/N (%)			
55-65				
65-75				
75+				
p-value				

Table 7. Performance Characteristics by Sex

Gender	Sensitivity			Specificity
	CRC	AA	OA	
Female	n/N (%)			
Male				
No Answer				
p-value				

Table 8. Performance Characteristics by Ethnic Background

Ethnic Background	Sensitivity			Specificity
	CRC	AA	OA	
African American	n/N (%)			
Asian				
Hispanic / non-white				
White				
Other				
Prefer not to answer				
p-value				

Table 9. Performance Characteristics by Other Precancerous Adenoma Subtype

Subtype	OPA Sensitivity	Specificity
Category 3.0	n/N (%)	

Category 4.0		
Category 5.0		

Table 10. Performance Characteristics by Advanced Adenoma Subtype

Subtype	AA Sensitivity	Specificity
Category 2.1	n/N (%)	
Category 2.2		
Category 2.3		
Category 2.4		

Table 11. Performance Characteristics by Cancer Stage

Stage	CRC Sensitivity	Specificity
Stage 0	n/N (%)	
Stage I		
Stage II		
Stage III		
Stage IV		

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.

4. Registration Procedures

4.1 Methods for Engagement

Initial study eligibility will be determined by an online screener provided by the CRO. The advertisement will inform participants about the study and provide basic information on what the study entails. Selection of the advertisement will direct participants to the survey landing page. Gastroenterologists and other individuals on this protocol can also refer participants to this survey landing page. The survey is a series of questions that preliminarily determines if the participant is eligible for the study. The survey interface provides the participant with one question at a time and can be visualized on any computer or smartphone.

4.2 Pre-Engagement

To recruit the patient volume and demographic representation needed for this study, patient identification efforts have been conducted prior to the official enrollment period. Engagement with human subjects during pre-enrollment was regulated under a previously approved IRB (Pro00032825). Beginning in August 2020, participants arrived at a survey landing page for the study and participants had the option to answer a series of questions collecting qualifying information for a follow up phone call from a call center. Patients who qualified for this follow up phone call were contacted to confirm their responses to the online survey, as well as their permission to receive communications leading up to, and during, the enrollment period.

The CRO recruited patients through advertisements placed on social media platforms. The advertisements informed participants that a clinical study is seeking eligible participants for future enrollment. Selection of the advertisement directed participants to the survey landing page and provided the participants with questions assessing future eligibility. If a participant was disqualified at this time, then the participant was redirected to a thank you page with no further planned communication. However, if a subject qualified, the participant was redirected to a thank you page, received a thank you email, and received a call from a representative with the CRO call center. The qualification and interest of participants was confirmed during this call, which triggered a series of communications that occur prior to the enrollment period. These communications include emails, text messages, newsletters, phone calls and other methods of electronic communication, as required. Participants are always provided an opportunity to opt-out of further communications at any point during this pre-enrollment phase. Once enrollment begins for this study protocol, participants will receive further details about the study and have an opportunity to provide verbal consent for continued participation.

During the previous study (Pro00032825), there were some participants who were not qualified or not able to participate at the time, however they expressed interest in receiving communications about future studies. These subjects will enter the same communication workflow described above.

4.3 Enrollment onto the study

During the official enrollment period, for participants who are considered eligible for the study based on the initial questionnaire and follow-up conversation, the call center at the CRO will contact the participant. The participant will be asked to confirm their identity against the details provided in the online screener by providing their full legal name and date of birth. During this call, the study member will share additional information about the study, including a copy of the Informed Consent Form (ICF) via email and / or text message. The participant will also be asked whether or not they have had sufficient time to read, understand and ask questions about this study and ICF. The participant may take as much time as they would like to read, understand and ask questions about this study and ICF. Additionally, the participant may ask the study member to read the entire ICF over the phone. During enrollment, a study member will discuss the risks and benefits of the study, answer any questions the patient may have about participation, and collect additional details for eligibility.

If the participant decides to enroll onto the study, the participant will consent to enrollment using a secure online signature platform. In the online signature platform, the participant will be asked to sign the informed consent form (ICF) as well as sign required medical data release forms for releasing colonoscopy and histopathology results. A participant will be considered enrolled after understanding and signing the ICF.

During enrollment, the subject will be assigned a unique subject identifier number that will permit deidentification of the subject through the entirety of the study. Whether the participant chooses to provide consent to participate or declines to participate, the participant's response will be recorded and saved for study documentation in the Trial Master File and subject to the Data Management Plan.

4.4 Confirming a Colonoscopy

This study requires eligible participants to have a routine, standard-of-care colonoscopy. After a participant has been enrolled onto the study, the study member will assist the participant to schedule a colonoscopy. Once the call

center confirms the subject has a colonoscopy scheduled, the study member will record this information and provide updates to the participant, as needed. The care coordinators at the call center will provide reminders to participants via email, text, or outbound phone calls to attend their appointments and to follow their physician's instructions in order to help them maintain eligibility.

5. Protocol Plan

5.1 Sample Collection

Once a participant has signed the ICF and scheduled a colonoscopy procedure, the study members at the CRO will issue the mt-sRNA test collection kit to the patient's residence. This collection kit will contain all materials necessary to collect a viable sample for processing. The Collection Kit will contain the following:

- **Collection Kit Instructions For Use:** Instructions for using the Collection Kit
- **Attestation Form:** Form to attest that the sample was derived from the correct participant.
- **iFOB Sampling Bottle**: OC-Auto Micro 80 iFOB Test (iFOB) Sampling Bottle
- **iFOB Sampling Bottle Ziplock Bag:** plastic bag to prevent stool leakage during shipping
- **Stabilization Liquid**: preservative buffer for stool storage during shipping
- **Sample Container**: plastic bucket for stool sample collection
- **Container Lid**: plastic top for stool sample collection
- **Sample Labels**: stickers for the collection kit components
- **Seat Bracket**: plastic tool to hold Sample Container for stool sample collection
- **Shipping Box**: cardboard container for return of stool sample
- **Prepaid Shipping Label**: barcode for return shipping of stool sample
- **Label Package Seals**: stickers for closing the mailing container
- **Inner Ziplock Bag (Bag #1):** plastic bag to prevent stool leakage during shipping
- **Outer Ziploc Bag (Bag #2)**: plastic bag to prevent stool leakage during shipping
- **Absorbance Sheet**: sheet to absorb liquid if leaked outside of the container

The specimen must be collected prior to initiation of bowel preparation for the colonoscopy. Collection materials will be labeled with the patient's unique identifier so that the sample can be linked to the individual. The collection materials will also contain an attestation form so the patient can confirm the origin of the sample. Detailed instructions will be provided to the participant in the form of an Instructions for Use (IFU) booklet. Per the IFU, stool samples and iFOBT swabs will be collected by the participant at the participant's convenience, so long as the collection was performed prior to the colonoscopy preparation and the colonoscopy procedure.

5.2 Collection Kit: Obtaining a stool sample and iFOBT swab

Once the collection kit is sent to the participant's house, the subject will be instructed to follow the collection kit IFU booklet. The collection kit IFU will provide step-by-step instructions with associated images for how to complete sample collection. Briefly, these instructions are provided below:

1. The patient will open the collection kit by ripping the perforated edge and opening the flaps of the box,
2. The patient will unpack the kit by placing the contents of the kit onto a surface near the stool collection location. This includes:
    a. Removing the sample container / lid from the box,
    b. Removing the seat bracket from the box,

   c. Removing the iFOBT tube from the box,
   d. Removing the Stabilization Buffer from the box,
3. The patient will collect the stool sample by placing the bracket onto the toilet seat and setting the sample container into the seat bracket. The patient will then replace the toilet seat and sit on the toilet. Finally the user will produce a stool sample into the sample container.
4. The patient will use the iFOBT Sampling Bottle to swab the stool sample. This requires:
   a. Unscrewing the iFOBT Sampling Bottle tube cap,
   b. Swabbing the stool sample in a cross-hatch motion using the swab on the iFOBT tube cap,
   c. Replacing the iFOBT tube cap into the iFOBT Sampling Bottle with the iFOBT buffer.
5. The patient will stabilize the stool sample by pouring the Stabilization Liquid into the stool sample container.
6. The patient will secure the sample by tightly sealing the sample container. This requires:
   a. Removing the sample container from the toilet and plating onto a steady surface,
   b. Placing the container lid onto the sample container,
   c. Pressing firmly onto all sides of the container lid until no more audible clicks can be heard,
   d. Rotating the lid to ensure proper seal.
7. The patient will be instructed to record sample information on the iFOBT Sampling Bottle and the Attestation Form.
8. The patient will place the components into the appropriate bags and all bags will be tightly sealed:
   a. iFOBT Sampling Bottle will be placed into small Ziplock Bag
   b. Sealed sample container will be placed into Ziplock Bag #1
   c. Ziplock Bag #1 will be placed into Ziplock Bag #2
9. All components will be placed into the shipping box. Components include:
   a. iFOBT Sampling Bottle in Ziplock bag,
   b. Sample container in two Ziplock bags,
   c. Attestation Form
   d. Empty Stabilization Buffer
10. The patient will be instructed on how to ship the sample back to Geneoscopy's Laboratories. This includes sealing the collection box and returning it to the FedEx drop off location or ordering a courier to pick up the box from the patient's residence.

If the subject encounters any issues during sample production, the participant will be instructed to call the CRO call center. If a collection kit is defective, the participant will be instructed to ship the collection kit back to Geneoscopy's centralized laboratory and a subsequent collection kit will be issued to the participant. The same rules should apply in the event that a stool collection kit is misplaced, lost, or never received.

Once the sample collection is complete, per the IFU, the participant is instructed to schedule a pickup or drop the collection kit off at a courier location. The return label on the collection kit will send the kit to Geneoscopy's centralized laboratory. The shipment and arrival to Geneoscopy will be tracked by the courier and will be recorded by the CRO. Once the sample arrives at Geneoscopy's laboratory, it will be assessed for viability (e.g., enough stool, damaged collection kit, etc.). If the stool sample does not meet the predefined requirements, the CRO will be immediately notified using an online portal. The CRO will contact the subject to request another stool specimen. If collection can occur prior to the participant's colonoscopy appointment, a subsequent collection kit will be shipped to the participants residence. If a kit cannot be issued in time, the study member will offer to reschedule the colonoscopy for the participant. If the participant is unwilling to reschedule the colonoscopy or if the participant does not wish to provide a subsequent stool sample, the patient will be considered ineligible and will be withdrawn from the study.

5.3 Sample Assays

Samples will be shipped in the mail at ambient temperature for up to 4 days. Samples may be stored at -80 degrees C as eukaryotic cell fractions and as total extracted nucleic acid. Trained technicians at the laboratory will be blinded to results of the colonoscopy. Care will be taken to ensure that the proper unique patient identifier is associated with all results.

Samples will be assessed to ensure they are sufficient for testing. If the sample arrives in a condition that is insufficient for testing, the CRO will be notified and the issuance of a subsequent kit will be attempted. If this is not possible, the subject's data will be deemed unevaluable and will not be used in the analysis. Samples that fail quality assessment will be noted and assessed by the Sponsor.

For samples that are processed, the assay result for the mt-sRNA test will be "positive", "negative", or "no result". A "no result" will be reported if a step in the analytic process fails to meet the quality metrics of the manufacturer. This can occur based on a control failure, a failure of RNA quantification, a failure of the hemoglobin assessment, or other process failure. Samples with a "no result" label will not be included in the study analysis.

5.4 Evaluation of a iFOBT test results

Once a collection kit is received by laboratory technologists at Geneoscopy, the iFOBT test will be evaluated using the iFOBT OC Auto® Micro 80 Analyzer. The output from this instrument is a continuous value, which indicates the concentration of hemoglobin in the stool sample. Geneoscopy will use a threshold value such that if the output from the iFOBT OC Auto® Micro 80 Analyzer is greater than the threshold, then the iFOBT test will be positive. If the output from the iFOBT OC Auto® Micro 80 Analyzer is less than the threshold, then the iFOBT test will be negative. The binary iFOBT result (positive or negative) is used as input into the mt-sRNA test software to determine the composite result for the diagnostic.

5.5 Analysis of seRNA in stool samples

In parallel with the iFOBT test analysis, the stool sample will be processed. Specifically, the stool sample will be homogenized, and approximately 45 mL of the homogenate will be aliquoted into 50mL conical tubes. The homogenate will be subjected to differential centrifugation using a tabletop centrifuge and the supernatant will be discarded. The pellet will be lysed using a thiocyanate guanidine-based lysis buffer. The lysate will be subjected to further centrifugation and filtration to preferentially isolate the eukaryotic nucleic acids. The layer enriched with human nucleic acid is used for nucleic acid extraction (**Figure 1**).

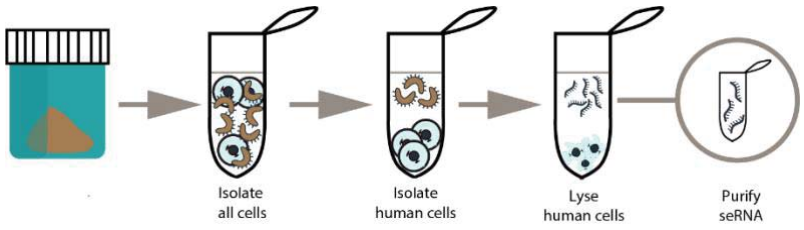

Figure 1. Methods for manual human nucleic acid enrichment using differential centrifugation.

Total nucleic acid isolation will be performed on the purified seRNA using the bioMérieux EMAG as per the manufacturer's instructions. The output from the EMAG is subsequently exposed to DNase treatment for removal of DNA contaminants that impact assay expression. Subsequently, the solution is subjected to a cleanup protocol using the bioMérieux EMAG as per the manufacturer's instructions.

Each sample will be evaluated for seRNA targets. The seRNA targets are quantified using Bio-Rad's QXDx ddPCR System (510(K) K181661). Evaluation of each seRNA biomarker requires use of a single assay, which consists of two primers and a fluorophore labeled probe. The probe can be labeled with FAM, HEX, or VIC. Assays can be duplexed such that two assays (four probes and two different fluorophores) are added to each well that is analyzed by the QXDx ddPCR system. The output from the QXDx system is a quantification of the concentration of all measured markers in the sample (**Figure 2**).

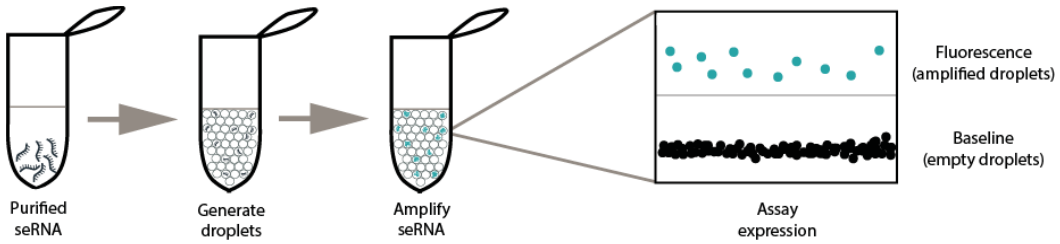

Figure 2. Methods for digital droplet PCR development and output for assessing quantification of human nucleic acid in purified RNA.

5.6 Generation of a Test Result

The software component will store, query, and process the required features (iFOBT test result, demographics, and RNA quantification) to determine an output for each mt-sRNA test (positive, negative, or no result). Input data required for the software will be stored in an online cloud-based storage unit. Using the software, data will be queried and transformed to generate the input layer for assessment by the model. Pushing the data through the model will generate a composite score for each individual patient. This score is calculated by multiplying the patient's individual features (expression, demographic information, and stool hemoglobin assay (iFOBT) results) by a predetermined constant marker-specific weighting factor. The aggregate of these individually weighted marker results determines the composite score, whereby a higher output score indicates that the patient has a higher risk of having an advanced adenoma (AA) or colorectal cancer (CRC). The composite score will be compared to a predetermined threshold. If the composite score is above the predetermined threshold, then the result will be reported as positive. If the composite score is below the predetermined threshold, the result will be reported as negative. If a score cannot be computed for any reason, the software will report "no result". The final output from the software is a patient report that will indicate the result from the mt-sRNA test.

5.7 Conducting a colonoscopy

After stool samples collection, the subject will undergo a routine, standard-of-care screening colonoscopy. The CRO will provide the subjects with reminders to attend their colonoscopy and to follow the instructions of their physician. Once the subject confirms the appointment, the participant will be instructed on how to prepare for a colonoscopy by the gastroenterologist at the endoscopy site. Consent and instructions for the colonoscopy procedure will also be provided by the endoscopy site. The night prior to the colonoscopy, the participant will prepare for the procedure according to the endoscopy site's instructions.

During the procedure, if the physician observes any findings, these findings will be reported, as per site-specific standard of practice. If lesions are removed during the procedure, the lesion will undergo histopathology review, as per normal practice of each clinical site. If the participant had no findings during the colonoscopy, the participant will not have associated histopathology.

The endoscopist will record the quality of the colonoscopy procedure. Quality metrics will include bowel preparation, colonoscopy withdrawal time, and procedure completion. If the quality of the colonoscopy was limited in any way, but the endoscopist observed a colorectal cancer or an advanced adenoma, then the participant will be eligible for the study. If the quality of the colonoscopy was limited but the colonoscopist did not observed an advanced adenoma or a colorectal cancer, then the subject can be considered eligible for the study. If the participant does not wish to receive a subsequent colonoscopy or the participant does not meet the aforementioned criteria, then they will be deemed ineligible for the study. Poor quality can include:

- Poor bowel preparation is poor (i.e., large amounts of stool, <90% of surface could be examined, or lesions <5mm could not be observed),
- Incomplete colonoscopy (did not reach the cecum, or small / large intestinal junction, or neo-cecum)

During this process, the CRO will contact the subject to find out whether they attended their appointment and if they have any questions about their participation in the study. If it is learned that the appointment was not attended, the CRO will communicate with the subject about the need to reschedule and collect new appointment details in order to maintain eligibility. These communications will come in the form of texts, emails, and outbound calls.

5.8 Obtaining clinical information, demographics and identification

Copies of subjects' medical records related to their colonoscopy are required to conclude participation in this study. Medical Record Release Forms will be signed during enrollment via a secure signature platform. Subjects will complete a medical record release form for each doctor involved in the recommendation and completion of their routine, standard-of-care, screening colonoscopy visit. Completed Medical Record Release Forms will be used by the study team to proactively request necessary records from each doctor involved in the recommendation and completion of the subject's routine, standard-of-care, screening colonoscopy.

Medical records may be obtained from the participant's primary care physician, the gastroenterologist who performed the procedure, and/or the pathologist who reviewed tissue extracted during the endoscopy. Medical records may include the following:

- **Primary Care Physician Medical Records**
  - Participant Name
  - Participant Date of Birth
  - Gender
  - Sex
  - Age
  - Ethnic Background
  - Smoking Status
  - Pathology Center Information
  - Treating Physician Information
  - Date of procedures
  - Prescriptions
  - History and Physical Information

- **Gastroenterologist Medical Records**
  - Participant Name

- Participant Date of Birth
- Gender
- Sex
- Age
- Ethnic Background
- Smoking Status
- Endoscopy Center Information
- Treating Physician Information
- Colonoscopy Date
- Colonoscopy Time
- History and Physical Information
- Number of Lesions Removed
- Location of Lesions

- **Pathology Medical Records**
  - Participant Name
  - Participant Date of Birth
  - Gender
  - Sex
  - Age
  - Ethnic Background
  - Smoking Status
  - History and Physical Information
  - Pathology Center Information
  - Treating Physician Information
  - Number of lesions reviewed
  - Lesion Category
  - Lesion Size
  - Recommended Clinical Care

Records will be sent to study members at the CRO via a secure method (secure FAX, secure email, or mail). All members at the CRO are non-conflicted study members. Medical records will be reviewed by study members at the CRO prior to uploading to the CRO's centralized database. Subsequently, the data will be reviewed by internal pathologists that are contracted by the CRO. All pathologists are non-conflicted study members. The pathologists will record information about the colonoscopy and the histopathology to determine disease severity (**Table 1**). These data will be entered into the CRO's centralized database. Each record will be linked to a participant using a unique patient identifier (UPN). All subsequent data obtained or distributed will be linked to the UPN. All data will be subject to a Data Management Plan.

5.9 Categorization of patients based on colonoscopy / histopathology

The results from the colonoscopy and histopathology will be blinded to study members at Geneoscopy. These results will be obtained and stored by a non-conflicted entityin a secure and HIPAA compliant portal.

The entity will generate patient labels based on colonoscopy findings and subsequent histopathology, if applicable. Patient classifications will be performed based on a pathology review standard operating procedure (SOP) that is aligned with the College of American Pathologists Protocol for Examination of Excisional Biopsy Specimens From Patients With Primary Carcinoma of the Colon and Rectum version 4.1.0.0. Initially, pathologists will report the number of lesions identified on a colonoscopy and the colonoscopy quality. The number of lesions will be an integer and can include 0. If there were no lesions observed on a colonoscopy, the entry will be considered complete. If lesions were identified on a colonoscopy, for each lesion, classification will require evaluating lesion location within the colon (site), specimen integrity, size (mm), configuration, and histology. Colon site can include: cecum, ileocecal valve, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, rectosigmoid colon, rectum, other (specify), not specified. Specimen integrity can include: intact, fragmented, or not specified. Polyp size will be reported as an integer in millimeters. The polyp size will be derived from the colonoscopy report. If the colonoscopy report provides a value that is a range, the average value of the range will be reported. If the colonoscopy report provides a value that is descriptive (i.e., diminutive, or small), the size will be reported according to the pathology reporting SOP. Polyp configuration will be reported as pedunculated, sessile, or not specified. Histology will be reported as one of the following: carcinoma, carcinoma *in-situ*, adenoma with high-grade dysplasia, tubular adenoma, villous adenoma, tubulovillous adenoma, traditional serrated adenoma, sessile serrated adenoma, sessile serrated polyp, hyperplastic polyp, fecal matter, normal mucosa, lymphoid aggregate, hamartomatous polyp, lipoma, neuroendocrine tumor, sarcoma, lymphoma, or other. After information has been submitted for each lesion, the pathology review will be considered complete.

Pathology review will be performed by two independent reviewers and will be compared for consistency using an internal algorithm. If inconsistencies exist, a third reviewer will provide classification information and the third review will be compared to the two original reviews. If the third review agrees with one of the original reviews, this will be the ultimate pathology review for the patient. If all three reviewers disagree with the label, the principal investigator on the study will evaluate all information and generate a final review of the patient.

Once pathology reviews are finalized, patient categories will be generated based on the information provided in **Table 1**. Subjects will be categorized by the most clinically significant lesion. Lesion categories from highest severity to lowest severity include colorectal cancer, advanced adenomas, other precancerous adenomas, hyperplastic polyps, and then no findings on a colonoscopy. Patients with invasive carcinoma will be classified as Category 1. Patients with an advanced adenoma will be classified as Category 2. Patients with other non-advanced adenomas will be classified as Category 3-5. Patients with hyperplastic polyps or with no findings on a colonoscopy will be classified as Category 6. Subcategories will also be determined using **Table 1** based on number of lesions, lesion size, and histology. For patients with CRC, additional reports will be obtained to assist in CRC staging. For all CRCs, lesion location, size, and TNM stage will be determined and recorded.

If the participant does not have a cancer or an advanced adenoma and the colonoscopy and histopathology reports are not clear enough to generate a definitive pathology classification, the participant will be excluded from the study analysis. Reasons for this type of exclusion can include: a biopsy or excision was not performed, the specimen was lost or mislabeled, the specimen was destroyed during the excision process, traceability between the colonoscopy lesion and the histopathology reading is not maintained, or other circumstance where pathology classification is not possible.

Based on the colonoscopy and histopathology results, patient management will be completed by physicians at the endoscopy center. Results from the mt-sRNA test will not be provided to patients.

5.10 Stool sample archival

If available, replicate stool samples will be archived for research use. Samples will be deidentified and will be barcoded with the patient's unique identifier for tracking purposes. Specimens will be stored indefinitely or until the entirety of the sample is used. Samples can be stored at Geneoscopy's Laboratories or at a bank that is contracted by Geneoscopy.

5.11 Study Schedule

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

- Obtain information
- Determine initial eligibility
- Obtain 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 Collection Kit
- Schedule colonoscopy and/or obtain procedure information
- Enter all patient data into the CRO 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 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 participant'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 iFOBT
- iFOBT will be read
- Sample will be processed using the molecular component of the mt-sRNA test
- Mt-sRNA Test score is generate for the participant
- Study members determine if a repeat stool sample collection is necessary and arranges for subsequent collection kit to be sent to the participant'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

- The 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 CRO

- Data will be entered in the CRO's patient portal

Pathology review: Can be completed at any time

- Study member determines if a repeat colonoscopy is needed or if the participant 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

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

- Study member determines if a repeat colonoscopy is needed or if the participant is ineligible based on reports

6. Study Materials and Device Chain of Custody

6.1 Sponsor-provided materials

Geneoscopy will contract to manufacture Collection Kits for the study. The contractor will manufacture the kits and distribute kits to the patient once they are enrolled into the study. The contractor will also be responsible for reissuing kits to patients, as needed. Geneoscopy will provide material specifications and bill of materials to the contractor including labeling and packaging information.

6.2 Site-provided materials

The individual endoscopy sites will be responsible for ensuring all standard of care requirements for a screening colonoscopy are provided to the patient prior to the procedure. This includes colonoscopy preparation materials, consent forms for the colonoscopy procedure, garments for the procedure, or other items that are required.

6.3 Device Chain of Custody Tracking Procedures

To ensure documentation of the device during manufacturing and transit, records will be kept on all collection kits through the the CRO's portal. In addition to the unique identifier provided by the CRO, two additional unique identifiers will be associated with Collection Kit components. These labels will be affixed to the external box, the stool sample collection bucket, the iFOBT swab, and the attestation form. These labels will be in human readable and barcode format. Barcodes can be scanned once received by the laboratory to ensure receipt. These barcodes will be used to track return of kits following sample collection. Records associating collection kits with samples will be stored in an electronic database that is maintained by the CRO and the Sponsor. Attestation forms will be retained by the Sponsor to confirm data within the electronic database.

7. Potential Risks and Benefits

7.1 Risks

Stool Sample:

Risks of using the mt-sRNA test are similar to risks associated with other stool-based *in-vitro* diagnostics. Sample collection is performed in the participant's residence, which is comparable to normal methods for waste removal. Risks include misuse of the collection device, and subsequent erroneous result. While there are no known serious risks to collecting stool samples, there may be unknown risks.

Colonoscopy:
Undergoing a colonoscopy has known risks. These risks include gastric distress prior to or after the procedure, perforation of the intestines during the procedure, and/or bleeding during or after the procedure. These risks are disclosed to the participant by the gastroenterologist's office prior to the procedure.

Medical records:
There is a rare possibility that confidential information about the subject may be accidentally disclosed. This risk is minimized by protections described in the Confidentiality section below and will be managed by the Data Management Plan.

Privacy Risks:
There is a rare risk that genetic research data could be shared with unauthorized users. This would result in the subject being at risk of loss of the privacy of their health data. This risk is minimized by implementing protections such as sample deidentification, use of secure databases, and training for all clinical trial personnel.

7.2 Benefits

There is no direct benefit to subjects from their participation in the study although they may benefit from receiving a colonoscopy. Through this study, subjects will be guided through the colorectal cancer screening process and will therefore be up-to-date with current screening guidelines. It is our hope that, in the future, other people might benefit from this study as we will be able to develop improved diagnostics to detect and prevent colorectal cancer.

7.3 Participant Remuneration

The participant will be provided a $200 gift card for participating in the study. The participant will be paid once the participant completes the study. The participation will be considered complete after the following have been received:

- The genetic sample(s) (stool)
- The completed iFOBT test
- Completed Attestation Form
- Completed Medical Record Release Form(s)
- Medical/clinical information provided during enrollment
- Colonoscopy result with histopathology, if applicable

7.4 Study Oversight

The principal investigator listed on the study will be responsible for study oversight. The principal investigator, in conjunction with the Geneoscopy team, will be responsible for monitoring, auditing, IRB review, and regulatory inspection by providing direct access to study related source data. Any required modifications or changes in the scope of the study will be handled by submitting an amendment to the IRB. Geneoscopy will not work outside of the scope of the proposed study unless these amendments are approved by the IRB.

8. Data and Safety Monitoring

8.1 Data Management

Various data will be collected from the participant and stored in a secure centralized database. All data will be subject to the Data Management Plan. Data will be obtained from five sources:

Eligibility Survey: The responses to the eligibility survey will be obtained from the CRO using a secure API. The following data will be collected:

- If the participant has ever had a colonoscopy
  - Date of last colonoscopy
  - Outcome of last colonoscopy
- If the participant has a colonoscopy scheduled for the future
  - Date of future colonoscopy
- Participant's personal history of colon or rectal cancer
- If the participant had a previous diagnosis of disease that increases the participant's risk of colorectal cancer
- If the participant is currently exhibiting gastrointestinal distress
- If the participant has even been screened for colorectal cancer with method other than colonoscopy
  - If yes, what screening mechanism was used
  - If yes, when did this take place
- Participant's family history of colorectal cancer
  - If yes, which family members
- Gender
- Age
- Race / Ethnicity
- Smoking status
- Annual household income
- Insurance type
- Communication information (e.g., email, address, phone)

Collection Kit Tracking Information: Sample tracking information will be collected using a courier's online portal. The following data will be collected:

- Date and time that the Collection Kit was shipped to the participant's requested location
- Date and time that the Collection Kit was shipped from participant's requested location to Geneoscopy's Laboratories
- Communication information (e.g., email, address, phone)

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. The data will be collected includes:

- Information contained on / within the Collection Kit (e.g., reagent lots and expiration dates, sample IDs)
- Information written on the Collection Kit or collection kit contents that was provided by the participant (e.g., collection time / date, participant date of birth)
- Information on the Attestation forms

- Information written on the iFOBT

Sample Processing Information: During processing at Geneoscopy's Laboratories, data that is generated will be obtained and stored using a secure central database. This includes:

- Data related to sample accession, RNA extraction, and RNA quantification
- RNA concentrations for seRNA biomarkers
- iFOBT data (e.g., Sampling Bottle status, iFOBT test results)

Clinical Data: Participant medical reports will be obtained from the participant's physicians. These reports are:

- Colonoscopy reports
- Histopathology reports (Surgical Pathology)
- History and Physical
- Physician notes
- Patient labels from processing colonoscopy / histopathology reports

8.2 IRB Review, Ethics, and Informed Consent

The protocol, informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. In addition, any subject recruitment materials must be approved by the IRB prior to being used. This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice and applicable regulatory requirements. Geneoscopy must submit any change to the protocol to the IRB for review and approval before implementation. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately and the reviewing IRB are notified within 10 working days.

It is the responsibility of the investigator to provide each subject with full and adequate verbal and written information using the IRB approved informed consent document, including the objective and procedures of the study and the possible risks involved before inclusion in the study. Verbal informed consent must be obtained prior to performing any study-related procedures, including a screening colonoscopy. A copy of the informed consent document must be provided to the study subject.

Investigators must provide a *curriculum vitae* (CV) to the IRB for review of credentials. The investigator and required study members must also provide the following, as needed:

- Financial disclosures
- Medical license, as needed
- Correspondence to IRB

8.3 Confidentiality

To help protect confidentiality, samples will not have any name or address on it; they will be labeled with a code in order to protect health information and identity. The specimen(s) will be stored in freezers in a locked laboratory at Geneoscopy's Laboratories. All coded specimens will be linked to study participants on a separate master list, which will be stored in a locked cabinet (the study coordinators have the only key), in a password protected file, or in a secure database. Specimens can be stored indefinitely. If a report or article about this study is written or the study data are shared with others, we will do so in such a way that participants cannot be directly identified.

8.4 Intended Use of Data

This study will work towards validating a non-invasive screening test that will assess risk for colorectal neoplasias. The added benefits to society will be to increase patient compliance, reduce the cost of healthcare, and provide better healthcare for patients.

8.5 Safety Monitoring

There are no known risks to collecting stool samples, though there may be unknown risks. If subjects do experience an Unanticipated Adverse Device Effect (UADE) during the collection of the stool sample, they are prompted to contact the study team as soon as possible. A UADE is any serious adverse effect on health or safety, 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 application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Non-serious Adverse Events (concerns, complaints, etc.) will also be recorded and maintained by the the CRO call center. Complaints will be entered into the online portal by the study member who receives the complaint.

8.6 Record Retention

The Sponsor and all associated parties must maintain records for at least 2 years after the termination of the investigation. Written approval from the Sponsor must be obtained prior to destruction of any study records. All sites are subject to investigation by the Sponsor, or other regulatory agencies that inspect study records. These inspections are meant to verify adherence to all study protocols.

8.7 Protocol Deviations

A deviation is defined as a change, departure, or alteration to the procedures of the research protocol that diverges from the procedures that are approved by the IRB. Upon discovery of a deviation, the Principal Investigator is responsible for reporting the deviation to the IRB, as required. Deviations must be entered into the online portal and reporting associated with deviations must be assessed during assessment of primary and secondary endpoints.

9. References

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