SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Stool RNA-Based Colorectal Cancer Screening Test
Device Trade Name:	ColoSense
Device Procode:	SBB
Applicant's Name and Address:	Geneoscopy, Inc. 2220 Welsch Industrial Court St. Louis, MO 63146
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P230001
Date of FDA Notice of Approval:	May 3, 2024

Breakthrough Device: Granted breakthrough device status on January 10, 2020

II. INDICATIONS FOR USE

ColoSense is a qualitative *in vitro* diagnostic test intended for the detection of colorectal neoplasia associated RNA markers and for the presence of occult hemoglobin in human stool. ColoSense is for use with the ColoSense Collection Kit, the ColoSense Test Kit, the ColoSense Software, and the following instruments: Polymedco Immunochemical Fecal Occult Blood Test (iFOBT) Analyzer; bioMerieux EMAG Nucleic Acid Extraction System; and Bio-Rad QXDx Droplet Digital Polymerase Chain Reaction (ddPCR) System. ColoSense is a single-site test performed at Geneoscopy, Inc.

A positive ColoSense result may indicate the presence of colorectal cancer (CRC), advanced adenomas (AA) or serrated precancerous lesions (SPL) and should be followed by a colonoscopy. ColoSense is indicated as a screening test for adults, 45 years of age or older, who are at average-risk for developing CRC. ColoSense is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

III. CONTRAINDICATIONS

ColoSense is NOT clinically indicated for use for patients that have the following:

- Personal history of colorectal cancer, adenomas, or other related cancers.
- Positive result from another colorectal cancer screening method within the last 6 months, or:
    - 12 months for Fecal occult blood test (FOBT) or fecal immunochemical test (FIT)
    - 36 months for FIT-DNA Test
- 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 (FAP)
    - Family history of colorectal cancer
    - Other hereditary cancer syndromes including but not limited to:
        - Hereditary non-polyposis colorectal cancer syndrome (HNPCC) or Lynch Syndrome, Peutz-Jeghers Syndrome, MUTYH Polyposis (MAP), Gardner's Syndrome, Turcot's (or Crail's) Syndrome, Cowden's Syndrome, Juvenile Polyposis, Cronkhite-Canada Syndrome, Neurofibromatosis and Familial Hyperplastic Polyposis

IV. WARNINGS AND PRECAUTIONS

- Patients should **NOT** provide a sample for the ColoSense test if you are experiencing any of the following:
    - Bleeding hemorrhoids
    - Bleeding cuts or wounds on hands
    - Rectal bleeding
    - Menstrual bleeding (period)
    - Diarrhea
- No colorectal cancer screening test is perfect. ColoSense may produce false negative or false positive results. Based on the clinical validation data for ColoSense, with 25/27 colorectal cancers from typical average risk patients identified (92.6%) and with a 95% two-sided confidence interval lower bound of 75.7%, there is a chance that as many as 24.3% of patients with colorectal cancer may be missed by this test.
    - A positive test result is recommended to be followed up by a colonoscopy. A false positive (incorrect) result occurs when ColoSense produces a positive result even though the individual does not have colorectal cancer or precancerous lesion.

    - A negative test result does not guarantee the absence of colorectal cancer or precancerous lesions. A false negative (incorrect) result occurs when ColoSense does not detect a colorectal cancer or precancerous lesion even though a colonoscopy could find colorectal cancer or precancerous lesions.
- The performance of ColoSense has been established in a prospectively designed, blinded, cross-sectional, decentralized study. The benefits and risks of programmatic colorectal screening (i.e., repeated testing over an established period of time) with Colosense has not been studied. Non-inferiority or superiority of ColoSense sensitivity as compared to other recommended screening methods for colorectal cancer, advanced adenomas, or serrated precancerous lesions has not been established.
- Cross-reactivity was observed in analytical studies using specimens from subjects with IBS, breast cancer, gynecological cancers, prostate cancer, and lipoma.
- Samples must be received by the laboratory within 96 hours of collection to be suitable for testing. Ensure samples are shipped within 24 hours following collection.
- The Instructions for Use must be followed carefully for accurate results.
- Fecal samples should be treated as if they are potentially infectious.

V. DEVICE DESCRIPTION

ColoSense is a **m**ultiple-**t**arget **s**tool-RNA (mt-sRNA) based in vitro diagnostic (IVD) device designed to analyze a patient's stool for qualitative detection of colorectal neoplasia associated RNA markers and the presence of occult hemoglobin in human stool. ColoSense evaluates eight **s**tool-derived **e**ukaryotic ribonucleic acid (seRNA) markers [(Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Aminoacylase 1 (ACY1), Amphiregulin (AREG), TNF Receptor Superfamily Member 10B (TNFRSF10B), Cadherin 1 (CDH1), Egl-9 Family Hypoxia Inducible Factor 2 (EGLN2), Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), Suppressor of Mothers against Decapentaplegic (SMAD) Family Member 4 (SMAD4)] and an occult hemoglobin assay result fecal immunochemical test (FIT)/iFOBT. Quantification of each of these markers is used in conjunction with smoking status to assess if the patient is likely to have colorectal cancer, advanced adenomas, or serrated precancerous lesions. Based on combined results of the RNA markers, hemoglobin, and smoking status, a single ColoSense result is determined. ColoSense results are qualitative (i.e., a positive or negative result is reported).

The following components are required for the use of ColoSense and qualified by Geneoscopy:

ColoSense Collection Kit:
Patient instructions for use (IFU)
OC-Auto® Sampling Bottle (FIT/iFOBT) with stool collection device
Stool sample collection bucket

Sample container lid
Plastic seat bracket
ColoSense Sample Preservative
Absorbent sheet
Bag 1-Prevent leakage of sample
Bag 2- Extra barrier to prevent leakage of sample
Bag 3-OC-Auto Sampling bottle
Test attestation form
Shipping Box

ColoSense Test Kit containing:
CS Assay Mix 1, Detect seRNA analytes
CS Assay Mix 2, Detect seRNA analytes
CS Assay Mix 3, Detect seRNA analytes
CS Assay Mix 4, Detect seRNA analytes
CS lPTC, Control generating a low positive ColoSense score
CS BTC, Control simulating background signal
CS S-Mix, Mix containing DNA polymerase and dNTPs in a buffer for ddPCR
CS S-Script, Reverse transcriptase
CS R-Out, RNase inhibitor

Hemoglobin Assay Reagents
Modified IVD fecal occult blood test (Polymedco OC-Auto Micro 80 FOB Test).

Ancillary Materials and Bulk Assay Reagents:
Nuclisens easyMAG Lysis Buffer
Nuclisens easyMAG Extraction Buffer 1
Nuclisens easyMAG Extraction Buffer 2
Nuclisens easyMAG Extraction Buffer 3
Nuclisens easyMAG Magnetic Silica
RNase-free DNase I
100% Ethanol
RNA Clean and Concentrator-5 Kit
Nuclease-free water
Nuclisens easyMAG Disposable
50 mL conical tubes
1.5 mL microcentrifuge tubes
PCR strip tubes
Disposable spatula
60 mL bulb syringe
100µm cell strainer
Automated Droplet Generation Oil for Probes
QXDx Droplet Reader Oil Pack
DG32 Automated Droplet Generator Cartridges
Pipet Tips for the AutoDG system
ddPCR 96-well Plates

PCR Plate Heat Seal
PCR Plate Sticky Seal
OC-Auto Buffer
OC-Auto Latex Reagent
OC-Auto Wash Solution
OC-Auto Positive Control
OC-Auto Negative Control
OC-Auto Calibrator
OC-Auto Cuvettes
OC-Auto Instrument Cups
OC-Auto Thermal Paper

Device Instrumentation and Software
bioMerieux EMAG System for Nucleic Acid Extraction
Bio-Rad QXDx Droplet Digital RT-PCR
Polymedco iFOBT Analyzer

ColoSense Software:
A composite score is generated by the ColoSense Software based on the patient's normalized seRNA biomarker expression, smoking status, and stool hemoglobin assay results to generate a ColoSense test result. The composite score is then compared to a predetermined threshold. If the composite score is greater than or equal to the predetermined threshold, the result is reported as positive. If the composite score is below the predetermined threshold, the result is reported as negative. If a score could not be computed, for any reason, the software does not generate a result and the sample result is labeled as invalid. The final output from the software is a result that indicates the outcome of the mt-sRNA test (ColoSense).

Principles of Operation
The ColoSense Collection Kit is sent to the patient's residence after being prescribed by a Healthcare Provider. Stool samples are self-collected using the ColoSense Collection Kit, which includes patient instructions for use, a stool sample collection bucket, a toilet seat bracket, an absorbent sheet, an OC-Auto Sampling Bottle, a ColoSense Sample Preservative and a shipping box. The patient deposits a stool sample into the ColoSense Collection Kit and mails the ColoSense Collection Kit along with the attestation form that includes the patient's smoking history back to Geneoscopy's laboratory.

Once the stool sample is received, the stool sample is directed into each of 2 parallel workflows for analysis: (1) a fecal immunochemical test (FIT)/ immunoassay fecal occult blood test (iFOBT) which detects blood in stool by antibody mediated binding to hemoglobin (Polymedco OC-Auto Micro 80 FOB Test, with modified threshold for use with this test); (2) detection of RNA contained in the cells that are shed by colorectal cancer, advanced adenomas, or serrated precancerous lesions into the stool.

The ColoSense test evaluates eight seRNA biomarkers, GAPDH, ACY1, AREG, TNFRSF10B, CDH1, EGLN2, KRAS and SMAD4. Isolation and analysis of seRNA analytes requires three steps. First, the eukaryotic cells are isolated from the stool sample and a solution containing eukaryotic nucleic acids is generated via lysis. Next, the seRNA is isolated using EMAG protocols, DNase treatment and a spin column based cleanup kit per the manufacturers' instructions. Finally, expression of the seRNA biomarkers is quantified using the ColoSense Test Kit and the QXDx droplet digital PCR system.

For the assessment of hemoglobin in stool, a modified IVD FOB test is used. The ColoSense test uses the OC-Auto Sampling Bottle for sample collection and the Polymedco OC-Auto Micro 80 Analyzer for reading the FOB results The iFOBT result is used as an input into the ColoSense Software to determine the result for the ColoSense test.

The ColoSense software is used to aggregate data from the seRNA biomarkers, smoking status and FIT/iFOBT to generate a score. The score is compared to the predefined threshold, yielding a positive (abnormal), negative (not detected) or no result. The score is calculated by using the patient's individual seRNA expression, smoking Status and stool hemoglobin (FIT/iFOBT) result, each of which is a marker-specific weighting factor. The aggregate of these individually weighted marker results determines the composite score, which is then compared to a test cut-off to determine a positive (abnormal) or negative result. If the composite score is greater than or equal to the test cut-off value, the ColoSense result will be reported as positive, and the patient should be referred for a colonoscopy. If the score is below the test cut-off value, the ColoSense result will be reported as negative, and the patient will be considered eligible for continued screening at recommended intervals. The actual score will not be provided in the test report sent to the healthcare provider.

Result Interpretation

The ColoSense test operates as a two-tiered algorithm in which the FIT result and the molecular component both contribute to the overall score. If the FIT result is positive, then the marker-specific weighting factor is added to the score and the ColoSense test will yield a positive result. In the event of a negative FIT result, the molecular (RNA) component of the test is then evaluated and, if positive, yield a positive result for the test. If neither the FIT component nor the molecular component is positive, then the aggregate composite score will yield a ColoSense result as either 'Negative Result' or in the case the test was not able to produce a valid result, 'No Result'.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Recommended screening for colorectal cancer includes both invasive and non-invasive options. Invasive options include colonoscopy, flexible sigmoidoscopy, flexible sigmoidoscopy with FIT, and CT colonography.

Non-invasive screening options include stool DNA-based colorectal cancer screening test, guaiac-based fecal occult blood testing (gFOBT), fecal immunochemical test (FIT) and blood-based plasma DNA testing.

Colonoscopy is considered to be the most accurate screening tool.

Patients who have a positive or abnormal test by an invasive or non-invasive screening method, except for colonoscopy, warrant further investigation through conventional colonoscopy. A patient should discuss these alternatives with their Healthcare Provider to select the method that best meets the patient's needs, expectations and lifestyle.

VII. MARKETING HISTORY

The ColoSense device has not been marketed in the United States or any foreign country."

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

ColoSense is an in vitro diagnostic (IVD) test that uses a non-invasive stool collection process. Therefore, potential adverse events (AEs) caused by or related to testing with ColoSense are unlikely. As with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. The primary risk associated with the ColoSense test is a false positive or false negative result. Since all positive results should lead to a diagnostic colonoscopy, false positive results may lead to patients being referred to colonoscopy unnecessarily. In the instance of a false negative result on ColoSense, there is a possibility that a case of colorectal cancer, advanced adenoma, or serrated precancerous lesions could go undetected.

IX. SUMMARY OF NON-CLINICAL STUDIES

Nonclinical studies were completed by Geneoscopy to evaluate the analytical performance characteristics of ColoSense. The studies are described below.

A. Algorithm Development and Cut-Off Determination

The cut-offs and the algorithm for the multi-target stool RNA test (mt-sRNA, ColoSense) were established based on stool samples prospectively collected from patients prior to preparing for and undergoing CRC screening via colonoscopy. Additionally stool samples were retrospectively obtained from individuals who had

been diagnosed with CRC or AA through colonoscopy but had not yet been treated for disease. In total, 1,327 participants were evaluated for eight (8) stool-derived eukaryotic RNA biomarkers, FIT results and smoking status. Model performance was assessed through 5-fold internal cross-validation of the training set (n=939) and by using the model on a hold-out testing set (n=388). In total, there were 1,114 samples that were employed in the final model development. The optimal threshold corresponded to maximal Youden's J statistic, which was picked from all possible points on the segment of the ROC curve that was bounded by the constrained specificity.

B. Analytical Sensitivity: Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantification (LoQ)

LoB, LoD, and LoQ studies were performed for the molecular component of the ColoSense test based on guidance from the Clinical & Laboratory Standards Institute (CLSI) Standard: EP17-A2 (Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline). The LoB was determined by processing a minimum of 60 replicates of sample preservative buffer spiked with human genomic DNA prior to extraction and run through the test system. The LoB was calculated non-parametrically as the highest measurement observed within the 95% confidence interval for blank samples across two reagent lots. LoD and LoQ were determined by processing stool samples with RNA concentration values near the expected LoD/LoQ threshold. RNA concentrations were derived using a combination of human RNA and synthetic RNA to obtain required concentration values. LoD was calculated per marker using a classical analysis by testing a minimum of 40 replicates per marker in a dilution series near the expected LoD. LoQ was calculated per marker as the lowest concentration with ≤20% coefficient of variation (CV) based on testing a minimum of 40 replicates per marker in a dilution series near the expected LoQ. LoD and LoQ were assessed by evaluating the maximum measurand for each marker across two reagent lots. The data is shown in **Table 1.**

Table 1. Analytical Sensitivity Characteristics Summary

Performance Characteristic	Result
Limit of Blank	All RNA Markers: 0.53 copies/µL
Limit of Detection	All RNA Markers: 0.31 - 0.66 copies/µL
Limit of Quantification	All RNA Markers: 1.29 - 2.55 copies/µL

C. Contrived Specimen Functional Characterization Study

A study was performed to demonstrate equivalency between clinical specimens and contrived specimens using data from the LoD/LoQ studies. Clinical stool specimens were aliquoted into replicates. Replicate analysis was performed on markers with low-level concentrations at or around the LoD or LoQ. The LoD for individual markers was calculated using classical analysis. LoDs derived using

clinical specimens were compared to those derived using contrived specimens to ensure functional equivalency. Two types of contrived specimens were utilized, one composed of synthetic RNA (SYN) templates diluted to achieve the appropriate concentrations for determination of the LoD for each marker, and a second composed of RNA extracted from pooled clinical stool samples, spiked with RNA to achieve concentration s for determination of the LoD for each marker (SM). Probit curves were generated for each marker and parsed by sample type. Across most markers, the 95% LoD confidence intervals for the clinical specimen probit curves overlapped with the confidence intervals generated from the contrived specimens (SYN and SM). The study results indicated that clinical specimens perform comparably to contrived specimens for the LoD/LoQ studies.

D. Analytical Specificity

1) In-silico Analysis of Primers and Probes

The potential for off-target binding of ColoSense primers and probes of the molecular targets of the ColoSense test was evaluated using publicly available *in-silico* analysis. Off-target binding was evaluated for human, enterobacterial, and viral transcripts. Study results found no potential for off-target amplification or binding from human, enterobacterial or viral non-specific targets.

2) Cross-Reactivity and Specificity

The potential for cross-reactivity with non-colorectal cancers and inflammatory conditions was evaluated by testing 131 subjects across 14 different diseases (**Table 2**). Subjects with known disease status provided a stool sample, which was assessed with the ColoSense test (FIT and molecular component) to generate ColoSense scores. False positive results were extrapolated by disease incidence to assess the impact of disease on ColoSense specificity.

Table 2. ColoSense Positivity Rates for Non-CRC Diseases and Cancers

Disease[a]	Number of samples tested	Incidence rate per 10,000[a]	% ColoSense Positive	Number of positive ColoSense calls in 10,000 patients
Bladder Cancer	4	1.87	0	0.0
Breast Cancer [d]	24	6.41	25.0	1.6
Esophageal Cancer	2	0.42	0.0	0.0
Gynecological Cancer[b, ,d]	6	4.89	50.0	1.2
Inflammatory Bowel Disease	8	1.3	12.5	0.2
Lung Cancer	6	5.2	0.0	0.0
Lupus	14	0.62	21.4	0.1
Pancreatic Cancer	1	1.11	0.0	0.0

Disease[a]	Number of samples tested	Incidence rate per 10,000[a]	% ColoSense Positive	Number of positive ColoSense calls in 10,000 patients
Prostate Cancer [d]	19	11.27	31.6	1.8[d]
Rheumatoid Arthritis	9	9	11.1	1.0
Celiac Disease[c]	2	1.26	50.0	0.6
Irritable Bowel Syndrome[c]	10	1,100	40.0	440.0
Lipoma[c]	19	21	15.8	3.3
Neuroendocrine Tumors[c]	7	0.37	0.0	0.0
Total per 10,000 patients				**5.9**

[a] Information was obtained from National Cancer Institute (http://seer.cancer.gov) and Center for Disease Control and Prevention (http://www.cdc.gov)

[b] The sum of all gynecological cancers was used.

[c] Data for these disease states were not part of the protocol and were identified incidentally; data was for informational use only.

[d] Value adjusted to represent sex-specific disease state.

The results of this study show a cross-reactivity of the test with irritable bowel syndrome (IBS), breast cancer, gynecological cancers, prostate cancer, and lipoma specimens. Based on the results of this study, considering the non-CRC diseases and cancers where the percent positivity was higher than would be expected in a normal population, the expected positivity for the tested diseases may result in potential false positive results in patients with IBS, breast cancer, gynecological cancers, prostate cancer, and lipoma, and average of six positive calls per 10,000 screening patients tested across all tested diseases. The test may be contraindicated for these patients.

3) Interfering Substances

Common substances that could be present in a stool sample were tested to assess potential interference with the molecular component of the ColoSense test. Clinical stool sample replicates were used to test the impact of an interfering substance on the ColoSense score. Clinical stool samples were derived from subjects with positive and negative ColoSense scores. For each interfering substance that was tested, 8 sample replicates were used to represent a range of ColoSense scores (positive and negative). In total, 28 substances were evaluated from the following categories:

- Common lotions, creams, and feminine OTC products
- Stool softeners, anti-diarrhea and laxative products
- Antacids and upset stomach remedies
- Common edible animal products

- Urine and alcohol
- Common vegetables and fruits
- Fecal fats

Testing was performed by spiking clinical stool sample replicates with the applicable substance. A ColoSense score was generated for the spiked stool sample, and that result was then compared to the result from an unspiked stool sample. Comparisons of the spiked group vs. the unspiked group for each substance tested found no statistically significant differences and no impact to ColoSense score. No interference was observed for the molecular component of the ColoSense test for any of the tested substances.

E. Carry-Over and Cross-Contamination Testing

1) Carryover

The objective of the carry-over study was to determine the potential for carry-over contamination for the molecular component of the test when ColoSense scores of negative samples are run immediately after high positive samples. Two synthetic RNA mixtures, that targeted a low negative (LN) ColoSense score and a high positive (HP) ColoSense score were evaluated. A plate containing 22 replicates of only LN mixture was run first as a reference plate. The LN plate run was followed by 2 plates containing 22 replicates of only HP mixtures. A final plate containing 22 replicates of only LN mixtures was run last in the sequence [plate #1 = LN1, plate #2 = HP1, plate #3 = HP2, plate #4 = LN2]; each plate additionally contained a blank template control (BTC) as well as a low positive template control (lPTC)] to assess carry-over. The study was conducted by 2 operators using 2 lots of ColoSense Test Kits on 2 QxDx droplet digital PCR (ddPCR) systems. Each operator ran 4 plates (LN1, HP1, HP2, LN2) using a unique lot of the ColoSense Kit and a unique QxDx test system over 4 days.

All plates were analyzed using the ColoSense Software to generate concentrations (copies/µL) of all ColoSense markers and ColoSense scores for each sample replicate. A t-test was used to determine whether there was a significant difference ($p<0.05$) in composite scores between the first LN plate (plate #1, LN1) and the last LN plate (plate #4, LN2). The acceptance criteria specified that for both lots of the ColoSense Test Kits, the ColoSense composite scores obtained from the negative samples in the last LN plate (LN2, plate #4) must not be significantly increased from the negative samples in the first LN plate (LN1, plate #1). The pre-specified acceptance criteria were met for both lots, as no carry-over was detected for the molecular component of the ColoSense test.

2) Cross Contamination

The objective of this study was to determine if cross-contamination occurs when performing the droplet digital PCR portion of the ColoSense test, synthetic RNA mixtures with low negative ColoSense scores were tested directly adjacent to synthetic mixtures with high positive ColoSense scores. The checkerboard plate

layout contained 11 high positive and 11 low negative samples along with a blank template control and a low positive template control on each plate. The study was conducted by 2 operators using 2 lots of ColoSense test kits on 2 QxDx systems over 3 test days. Two plates were used, plate #1 containing only synthetic RNA mixtures with low negative (LN) samples as a reference plate and plate #2 containing synthetic RNA mixtures containing high positive (HP) and low negative (LN) synthetic RNA mixtures in a checkerboard pattern. Acceptance criteria for both lots of the ColoSense test kits, required that the ColoSense composite scores obtained from the negative samples in the HP/LN plate #2 must not be significantly elevated (one-tailed t-test, $p<0.05$) from the negative samples in the LN plate #1. No significant difference was found in LN scores from either plate #1 or plate#2. Therefore, there was no significant cross-contamination for the molecular component of the ColoSense test.

F. Precision and Reproducibility – Molecular Component

Precision and reproducibility studies were conducted to assess variation of the ColoSense assay based on guidance from CLSI Standard: EP05-A3E (Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, Third Edition) and EP12-A2 (User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition). These precision studies were performed to validate that the test generates consistent results across different lots, runs and operators. The studies analyzed variance of the ColoSense test system within and between runs, operators, kit lots, and instrument systems for both the overall ColoSense score as well as each component of the ColoSense test system.

To evaluate variance of the overall ColoSense score, 4 score groups were evaluated:

- Low Negative (LN) = ColoSense score < 0.075
- High Negative (HN) = ColoSense score approximately 0.075 - 0.110
- Low Positive (LP) = ColoSense score approximately 0.120 - 0.170
- High Positive (HP) = ColoSense score > 0.180

Eight (8) stool samples per score group were evaluated. For each stool sample, a total of three (3) RNA replicates were assessed. Each of the three replicates were assessed on three (3) unique days, by three (3) unique operators, three (3) unique QXDx instrument combinations and three (3) unique lots of reagents.

Determination of precision based on each sample score group is noted in **Table 3**. Replicates of all samples were used to characterize the performance of the ColoSense score by examination of the variance between ColoSense results (%CV). The overall component of the ColoSense score was reproducible with all score levels having %CV less than 20%.

Table 3. Overall Precision for ColoSense Score

Score Group	# of Samples	Mean	Standard Deviation	%CV	# correct calls/ # all calls	% Correct Calls
Low Negative	8	0.076	0.0029	3.9%	24/24	100%
High Negative	8	0.100	0.0046	4.6%	24/24	100%
Low Positive	8	0.123	0.0046	3.7%	24/24	100%
High Positive	8	0.220	0.0118	5.4%	24/24	100%

ColoSense precision for operator-to-operator (**Table 4**), lot-to-lot (**Table 5**), and system-to-system **Table 6)** was assessed using pooled clinical specimens (stool samples) that generated ColoSense scores within the score groups listed above. Pooled stool samples without RNA spiking were used to generate replicates for the high negative group (HN). Pooled stool samples spiked with synthetic RNA were used to generate replicates for the remaining three (3) groups: low negative (LN), low positive (LP), and high positive (HP). Each score level was generated through two (2) independent preparations of the appropriate sample type (e.g., LN1 and LN2).

For each targeted score level, there were 18 results per lot per sample preparation and 54 results per sample preparation for the entire study. There were 144 results per lot of ColoSense reagents and 432 total results for the entire study (note: 2 replicates failed quantification and were not used for precision assessments).

Pooled stool samples without RNA spiking were used to generate replicates for the high negative group (HN). Mixtures of synthetic RNA transcripts were used to generate replicates for the remaining three (3) groups: low negative (LN), low positive (LP), and high positive (HP). For LN replicates, we observed 100% concordance with the original sample type between three operators **(Table 4**). For HN, LP, and HP replicates, we observed %CV ≤ 20% between three operators (range = 1.90% - 11.04%).

Table 4. ColoSense Precision (operator-to-operator)

Score Group	Operator	Mean	SD	Result	Acceptance Criteria	% (n/N)
Low Negative	Operator 1	0.045	0.001	100% concordance	Concordance > 95%	100% (36/36)
	Operator 2	0.046	0.009	100% concordance	Concordance > 95%	100% (36/36)
	Operator 3	0.044	0.001	100% concordance	Concordance > 95%	100% (36/36)
High Negative	Operator 1	0.100	0.002	1.90% CV	% CV ≤ 20%	100% (35/35)
	Operator 2	0.100	0.002	2.31% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	0.100	0.002	2.10% CV	% CV ≤ 20%	100% (36/36)

Score Group	Operator	Mean	SD	Result	Acceptance Criteria	% (n/N)
Low Positive	Operator 1	0.157	0.008	5.33% CV	% CV ≤ 20%	100% (36/36)
	Operator 2	0.155	0.007	4.41% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	0.154	0.006	3.93% CV	% CV ≤ 20%	100% (35/35)
High Positive	Operator 1	0.288	0.032	11.04% CV	% CV ≤ 20%	100% (36/36)
	Operator 2	0.274	0.017	6.24% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	0.279	0.019	6.08% CV	% CV ≤ 20%	100% (36/36)

Pooled stool samples without RNA spiking were used to generate replicates for the high negative group (HN). Mixtures of synthetic RNA transcripts were used to generate replicates for the remaining three (3) groups: low negative (LN), low positive (LP), and high positive (HP). For LN replicates, we observed 100% concordance with the original sample type between three reagent lots (**Table 5**). For HN, LP, and HP replicates, we observed %CV ≤ 20% between three reagent lots (range = 1.73% - 12.06%).

Table 5. ColoSense Precision (lot-to-lot)

Score Group	Operator	Mean	SD	Result	Acceptance Criteria	% (n/N)
Low Negative	Lot 1	0.046	0.009	100% concordance	Concordance > 95%	100% (36/36)
	Lot 2	0.044	0.001	100% concordance	Concordance > 95%	100% (36/36)
	Lot 3	0.045	0.001	100% concordance	Concordance > 95%	100% (36/36)
High Negative	Lot 1	0.101	0.002	2.17% CV	% CV ≤ 20%	100% (36/36)
	Lot 2	0.099	0.002	1.99% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	0.099	0.002	1.73% CV	% CV ≤ 20%	100% (35/35)
Low Positive	Lot 1	0.156	0.009	5.59% CV	% CV ≤ 20%	100% (35/35)
	Lot 2	0.155	0.005	3.17% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	0.155	0.008	4.86% CV	% CV ≤ 20%	100% (36/36)

Score Group	Operator	Mean	SD	Result	Acceptance Criteria	% (n/N)
High Positive	Lot 1	0.284	0.034	12.06% CV	% CV ≤ 20%	100% (36/36)
	Lot 2	0.275	0.013	4.73% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	0.281	0.019	6.84% CV	% CV ≤ 20%	100% (36/36)

Pooled stool samples without RNA spiking were used to generate replicates for the high negative group (HN). Mixtures of synthetic RNA transcripts were used to generate replicates for the remaining three (3) groups: low negative (LN), low positive (LP), and high positive (HP). For LN replicates, we observed 100% concordance with the original sample type between four systems (**Table 6**). For HN, LP, and HP replicates, we observed %CV ≤ 20% between four systems (range = 1.86% - 12.70%).

Table 6. ColoSense Precision (system-to-system)

Score Group	System	Mean	SD	Result	Acceptance Criteria	% (n/N)
Low Negative	System 1	0.045	0.001	100% concordance	Concordance > 95%	100% (24/24)
	System 2	0.045	0.001	100% concordance	Concordance > 95%	100% (36/36)
	System 3	0.045	0.001	100% concordance	Concordance > 95%	100% (24/24)
	System 4	0.047	0.011	100% concordance	Concordance > 95%	100% (24/24)
High Negative	System 1	0.100	0.002	1.86% CV	% CV ≤ 20%	100% (23/23)
	System 2	0.100	0.002	2.10% CV	% CV ≤ 20%	100% (36/36)
	System 3	0.100	0.003	2.60% CV	% CV ≤ 20%	100% (24/24)
	System 4	0.099	0.002	1.92% CV	% CV ≤ 20%	100% (24/24)
Low Positive	System 1	0.158	0.009	5.83% CV	% CV ≤ 20%	100% (23/23)
	System 2	0.155	0.007	4.72% CV	% CV ≤ 20%	100% (36/36)
	System 3	0.154	0.005	3.02% CV	% CV ≤ 20%	100% (24/24)
	System 4	0.154	0.006	4.08% CV	% CV ≤ 20%	100% (24/24)
	System 1	0.292	0.037	12.70% CV	% CV ≤ 20%	100% (24/24)

Score Group	System	Mean	SD	Result	Acceptance Criteria	% (n/N)
High Positive	System 2	0.277	0.018	6.32% CV	% CV ≤ 20%	100% (36/36)
	System 3	0.278	0.014	5.04% CV	% CV ≤ 20%	100% (24/24)
	System 4	0.274	0.021	7.68% CV	% CV ≤ 20%	100% (24/24)

Results from the Precision and Reproducibility studies met predefined acceptance criteria.

G. <u>Precision and Reproducibility – FIT/iFOBT</u>

FIT/iFOBT precision studies were performed using the OC-Auto Sampling Bottle (Polymedco) and OC-Auto Micro 80 Analyzer (Polymedco) to support the ColoSense cutoff. A reproducibility study was conducted to assess variation of the FIT/iFOBT component of the ColoSense test based on guidance from CLSI Standard: EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, Third Edition). This study analyzed variance of the iFOBT component of the ColoSense test system within and between runs, operators, OC-Auto Sampling Bottle lots, and instrument systems.

This study was performed at 1 site with 2 operators. Each operator performed a total of 6 runs using 3 lots of OC-Auto Sampling Bottles. Each run included 10 samples with 2 replicates of the following targeted hemoglobin concentrations spiked into a background stool matrix: low negative (LN), high negative (HN), low positive (LP), and high positive (HP).

Additional FIT/iFOBT analysis was performed which included testing each of the four (4) contrived score levels with 240 replicates. Each score level was tested 20 times using 12 distinct runs. Note that each sample was presented to the instrument 120 times and the sample was tested twice within a single run. This introduces a sample-to-sample comparison within each run as well as a pure repeatability component (between replicates from a given sample). The testing was performed by 2 operators using 3 lots and 2 systems.

Overall, the FIT/iFOBT component of the ColoSense test had a percent concordance for all score levels greater than 95% or %CV less than 20% (**Table 7)**. The FIT/iFOBT component of the ColoSense test results assessing operator-to-operator performance are shown in **Table 8,** the ColoSense test results showing lot-to-lot performance are shown in **Table 9** and the ColoSense Test results showing system-to-system performance are shown in **Table 10**.

Table 7. Overall FIT Precision

Precision Parameter	Target Level	Result: % Concordance / Max % CV	Acceptance Criteria	# Correct Calls / # All Calls	% Correct Calls
Within Laboratory	Low Negative	100% concordance	Concordance > 95%	240/240	100%
	High Negative	12.6 % CV	% CV ≤ 20%	225/240	93.8%
	Low Positive	9.3 % CV	% CV ≤ 20%	216/240	90.0%
	High Positive	10.6 % CV	% CV ≤ 20%	238/240	99.2%

Table 8. FIT Precision by Operator

Precision Parameter	Target Level	Operator	Result: % Concordance / Max % CV	Acceptance Criteria	# Correct Calls / # All Calls	% Correct Calls
Within Operator	Low Negative	Operator 1	100% concordance	Concordance > 95%	120/120	100%
		Operator 2	100% concordance	Concordance > 95%	120/120	100%
	High Negative	Operator 1	12.0% CV	% CV ≤ 20%	120/120	100%
		Operator 2	11.0% CV	% CV ≤ 20%	105/120	87.5%
	Low Positive	Operator 1	9.1% CV	% CV ≤ 20%	103/120	85.8%
		Operator 2	8.9% CV	% CV ≤ 20%	109/116	94.0%
	High Positive	Operator 1	11.1% CV	% CV ≤ 20%	118/120	98.3%
		Operator 2	8.8% CV	% CV ≤ 20%	120/120	100%

Table 9. FIT Precision by Lot

Precision Parameter	Target Level	Lot	Result: % Concordance / Max % CV	Acceptance Criteria	# Correct Calls / # All Calls	% Correct Calls
Within Lot	Low Negative	Lot 1	100% Concordance	Concordance > 95%	80/80	100%
		Lot 2	100% concordance	Concordance > 95%	80/80	100%
		Lot 3	100% concordance	Concordance > 95%	80/80	100%
	High Negative	Lot 1	12.3% CV	% CV ≤ 20%	73/80	92.2%
		Lot 2	12.3% CV	% CV ≤ 20%	79/80	98.8%
		Lot 3	12.9% CV	% CV ≤ 20%	73/80	92.2%
	Low Positive	Lot 1	10.7% CV	% CV ≤ 20%	70/80	87.5%
		Lot 2	9.3% CV	% CV ≤ 20%	67/80	83.8%
		Lot 3	7.2% CV	% CV ≤ 20%	75/76	98.7%
	High Positive	Lot 1	8.8% CV	% CV ≤ 20%	80/80	100%
		Lot 2	8.0% CV	% CV ≤ 20%	78/80	97.5%
		Lot 3	11.5% CV	% CV ≤ 20%	80/80	100%

Table 10. FIT Precision by System

Precision Parameter	Target Level	System	Result: % Concordance / Max % CV	Acceptance Criteria	# Correct Calls / # All Calls	% Correct Calls
Within Test System	Low Negative	System 1	100% concordance	Concordance > 95%	120/120	100%
		System 2	100% concordance	Concordance > 95%	120/120	100%
	High Negative	System 1	13.5% CV	% CV ≤ 20%	111/120	92.5%
		System 2	11.5% CV	% CV ≤ 20%	114/120	95.0%
	Low Positive	System 1	9.0% CV	% CV ≤ 20%	105/120	87.5%
		System 2	9.5% CV	% CV ≤ 20%	107/116	92.2%
	High Positive	System 1	11.2% CV	% CV ≤ 20%	118/120	98.3%
		System 2	9.1% CV	% CV ≤ 20%	120/120	100%

H. Robustness

The objective of the robustness study was to evaluate the impact of variations in the test procedure throughout the entire ColoSense test workflow The ColoSense procedure includes sample preparation, RNA extraction, and digital PCR analysis. Each section contains individual steps that are either manual, semi-automated, or fully automated. Items tested in the robustness study were taken from the manual and semi-automated portions of the ColoSense test workflow.

To assess robustness, stool samples were obtained from individuals with positive ColoSense scores and negative ColoSense scores. Stool samples were aliquoted into replicates to test variation in steps (low/high) relative to the expected (nominal) testing condition. For each variation that was tested, 8 sample replicates were used to represent a range of ColoSense scores (positive and negative). Variations tested included the following:

- Variations related to RNA extraction including amount of reagent added and timing for extraction steps
- Variations related to timing of digital PCR steps including duration of individual steps and time between steps

Variation was introduced for each step and the testing results generated after introducing variation were compared to the expected results. All samples showed %CVs of less than 5% (range = 0.003% - 4.84%) between the nominal and low/high condition replicates. All testing determined that variation in manual or semi-automated steps of the ColoSense test do not have any detectable effects on test performance.

I. Stability Studies

ColoSense Sample Preservative Stability
The objective of this study was to determine the shelf-life stability of the ColoSense Sample Preservative when stored at 15°C to 30°C. The Sample Preservative stability study was determined for the molecular component of the test by evaluating the performance of 3 sample preservative lots over a period of 15 months to support a 12-month shelf-life claim. Two lots of the Sample Preservative were stored at 30°C and 1 lot of the Sample Preservative was stored at 15°C. A total of 210 bottles (70 per lot) of Sample Preservative were used for the study.

Seven clinical stool samples x 2 replicates were tested per buffer lot at each timepoint (T0=within 30 days of manufacture, then at month 1, 2, 3, 6, 9, 12, 13, 14 and 15) Fourteen RNA replicates were evaluated per lot of Sample Preservative at each time point.

The acceptance criteria specified that ≥85% of the replicates generated from the stool samples must have ColoSense scores concordant with the T0 result to define stability. Additionally, GAPDH had to pass the threshold for detection as determined by the ColoSense software for ≥85% of all replicates at each timepoint. ColoSense scores were generated across all samples and timepoints and all samples used in the Sample Preservative stability study met the pre-specified acceptance criteria. The study demonstrated that the ColoSense Sample Preservative is stable through 12 months of testing when stored at a temperature of 15°C to 30°C.

Test Kit Long-Term Storage Stability
The objective of the study was to determine the shelf-life stability for all ColoSense Test Kit components when stored at -10°C to -30°C. The test kit storage stability was determined for the molecular component of the test using 3 ColoSense kit lots and 2 test kits from each lot were utilized per stability timepoint. Each lot was evaluated for stability using 2 plates (plate #1 and plate #2) at the following time points post time 0: (T1=1 month after date of manufacturing release, 2, 3, 6, 9, 10.5, 12 and 13 months after the date of manufacturing release) to support a kit stability claim of 12 months.

Samples used to evaluate the molecular test component were synthetic DNA controls provided as part of the ColoSense test kit and synthetic RNA mixtures required for quality control (QC) testing of the ColoSense test kit. At each time point, replicates of each control or mixture were tested. The samples used in the study included 7 replicates of the low positive template control (lPTC), 4 or 7 replicates of the blank template control (BTC), 6 replicates of QC RNA controls 1-3 and 4 replicates of QC RNA control 4. The acceptance criteria for plate #1 specified that ≥85% of BTCs must have concentrations for all markers below the accepted BTC level of 1 copy/µL. Additionally, ≥85% of the lPTCs must have

ColoSense scores within the established acceptable range of 0.125 and 0.180. The acceptance criteria for plate #2 specified that for each control mix ≥75% of wells with expected amplification must report greater than 20 copies/µL for both targets. Additionally, for each control mix, ≥85% of wells without expected amplification (i.e., wells that should not generate any target signal) must report copy numbers ≤0.5 copies/µL for both targets. Each lot was required to pass the acceptance criteria at the 13-month storage timepoint to claim a shelf-life stability at 12 months.

The ColoSense Test Kit met the acceptance criteria for storage stability for all kit lots out to 12 months. With test kit lot 2 not meeting the acceptance criteria at the 13-month time point, for expected amplification of the QC controls, the ColoSense Test Kit was determined to be stable for up to 10 months of storage at -10°C to -30°C.

Test Kit Freeze-Thaw Stability Study

The objective of the freeze-thaw (F-T) stability study was to determine the stability of all components of the ColoSense Test Kit when subjected to multiple freeze-thaw cycles, including the frozen condition at -10°C to -30°C and thaw condition at 15°C to 30°C.

Samples used to evaluate the molecular test component included a synthetic DNA control provided as part of the ColoSense test kit and synthetic RNA mixtures required for quality control (QC) testing of the ColoSense Test Kit. After each freeze-thaw cycle, replicates of each control or mixture were tested. Samples included 7 replicates of the low positive template control (lPTC) and blank template control (BTC), 6 replicates of QC RNA controls 1-3, and 4 replicates of QC RNA control 4. The test kit F-T stability was performed using 3 ColoSense Test Kit lots and 2 test kits from each lot were utilized per freeze-thaw cycle. Two plates (plate #1 and plate #2) were used for each testing cycle.

To execute the freeze-thaw cycle, test kits were removed from storage between -10°C to -30°C and thawed at room temperature for 30 minutes. Test kits were then returned to storage between -10°C to -30°C for a minimum of 12 hours before the next freeze-thaw cycle. Nineteen ColoSense test runs were conducted over 5 testing days by 3 operators. The acceptance criteria for plate #1 specified that ≥85% (6/7 replicates) of BTCs must have concentrations for all markers equal to orµ below the accepted BTC level of 1 copy/µL. Additionally,acceptance criteria specified that ≥85% (6/7 replicates) of lPTCs must have ColoSense scores within the established acceptable range of 0.125 and 0.180 and that the average concentration of the lPTC targets must be at least 10 copies/µL. The acceptance criteria for plate #2 specified that for each QC control mix, ≥75% of wells with expected amplification must report greater than or equal to 20 copies/µL for both targets. Additionally, for each QC control mix, acceptance criteria specified that ≥85% of wells without expected amplification must report copy numbers ≤0.5 copies/µL for both targets. Furthermore, each lot must pass

the acceptance criteria outlined above after 3 freeze-thaw cycles to claim stability after 2 freeze-thaw cycles.

The ColoSense Test Kit freeze-thaw stability study met he prespecified acceptance criteria for all 3 freeze-thaw cycles that were performed as part of the freeze-thaw stability study. The data demonstrated that the ColoSense test kit is stable for 2 freeze-thaw events.

FIT/iFOBT Stability

The objective of the OC-Auto Micro 80 iFOBT (FIT) stability study was to determine the stability of the collected FIT/iFOBT sample swab in the OC-AutoSampling Bottle when stored at 2°C to 8°C. This test provides a qualitative (positive/negative) result that is used along with the ColoSense test to generate a score.

FIT/iFOBT stability was determined by testing a total of 24 sample replicates contrived to contain hemoglobin concentrations above and below the positive test threshold. The samples consisted of 2 contrived stool pools with the following targeted hemoglobin concentrations: high negative (HN), target ~60ng/mL and low positive (LP), target ~90ng/mL. Each pool was swabbed 48 times with 1 lot of OC-Auto Sampling Bottles. Each pool was then swabbed 48 times with a second lot of OC-Auto Micro 80 Sampling Bottles. On the same day, 8 swabs from each lot and target level were tested on the FIT/ OC -Auto Micro 80 Analyzer (Day 0). The samples were stored at 2 °C to 8°C until testing at each timepoint (T0= day of pool generation and swabbing, then 7, 15, 23, 30 and 32 days).

Three replicates of each swab were tested totaling 96 replicates evaluated at each time point (8 swabs x 3 replicates x 2 lots x 2 target levels). The study was performed over the course of 32 test days by two operators on 1 OC-Auto Micro 80 Analyzer.

Acceptance criteria specified that hemoglobin concentration outputs must have ≤20% CV for each timepoint through 32 days. The OC-Auto Sampling Bottle met the stability acceptance criteria of ≤20% CV through 32 days for the high negative ad low positive samples for both OC-Auto sample bottle lots. The samples in the OC-Auto ampling bottles were determined to be stable for up to 30 days when stored at 2 °C to 8°C.

Animal Studies

N/A

Additional Studies

N/A

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

A clinical study was performed to generate data to support the safety and effectiveness of qualitative detection of colorectal neoplasia associated RNA markers and the presence of occult hemoglobin in human stool for the purpose of aiding in the detection of colorectal cancer (CRC) advanced adenomas (AA) and serrated precancerous lesions (SPL) with the ColoSense test. To evaluate the performance of ColoSense, the ColoSense test result was compared to the colonoscopy result and histopathologic diagnosis of all lesions discovered during colonoscopy. A summary of the clinical study is presented below.

A. Study Design

A prospective, blinded, cross-sectional, decentralized study (CRC-PREVENT) to evaluate ColoSense was performed, with a total of 14,263 participants enrolled in the study. Upon enrollment, participants were provided with a ColoSense collection kit to provide a stool sample for ColoSense testing. After providing a stool sample, subjects were required to undergo a screening colonoscopy within 120 days.

Subject stool samples were shipped to a single site laboratory for ColoSense testing. Stool samples were tested with ColoSense by laboratory technicians who were blinded to colonoscopy results. ColoSense results were compared to colonoscopy results, which were based on histopathological review of all lesions either biopsied or resected during the colonoscopy. Subjects that did not have any lesions identified were categorized as negative by colonoscopy. Performance was analyzed to assess whether there may have been any differences based on whether the colonoscopy was performed by community physician vs GI specialist and no difference in performance was observed. Histopathological results from biopsied tissue or excised lesions were categorized based on the most clinically significant lesion present by pathologists according to the pre-specified standards outlined in **Table 11**.

Table 11. Histopathological Categories

	Test Result	Category	Colonoscopy Findings
Colorectal Cancer (CRC)	Positive	1.0	Stage I-IV colorectal cancer, any size
Advanced Adenoma (AA)		2.1	High-grade dysplasia or ≥10 adenomas, any size
		2.2	Tubulovillous adenoma, any size
		2.3	Tubular adenoma, ≥10mm
Serrated Precancerous Lesions (SPL)		2.4	Sessile serrated lesions with dysplasia (SSLDs); Traditional serrated adenoma (TSA) (include size greater than 1 cm and/or presence of high-grade dysplasia); Conventional adenomas with serrated architecture; Sessile serrated lesions ≥10mm
Non-advanced Precancerous Lesions (NAPL)	Negative	3.1	5-9 adenomas or sessile serrated lesion, <10mm, non-advanced
		3.2	3-4 adenomas or sessile serrated lesion, <10mm, non-advanced
		4.0	1-2 adenomas or sessile serrated lesion, 5-9mm, non-advanced
		5.0	1-2 adenomas or sessile serrated lesion, <5mm, non-advanced
Negative Findings (NEG)		6.1	Hyperplastic polyp or negative lesions
		6.2	No lesions on colonoscopy

1. Clinical Inclusion and Exclusion Criteria

Enrollment in CRC-PREVENT study was limited to subjects who met the following inclusion criteria:

- Subject is male or female, ≥ 45 years of age
- Subject is able to understand the study procedures, 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 a stool sample
- Subject is able and willing to undergo a colonoscopy after providing a stool sample

Subjects were not permitted to enroll in the CRC-PREVENT study if any of the following exclusion criteria was met:

- Subject had any precancerous findings on most recent colonoscopy
- Subject has a history or diagnosis of colorectal cancer
- Subject has a history or diagnosis of aerodigestive tract cancer
- Subject had 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 had a colonoscopy in the previous nine (9) years
- Subject 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" or "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, or Familial Hyperplastic Polyposis

2. Clinical Endpoints

Primary Endpoints

Performance characteristics (sensitivity and specificity) of ColoSense were analyzed based on predefined acceptance criteria:

- With regards to the ColoSense sensitivity for subjects with CRC, the sensitivity of CRC must be greater than or equal to 90% and the lower bound of the 95% two-sided confidence interval must be greater than or equal to 80%.
- With regards to the ColoSense sensitivity for subjects with AA, the sensitivity of AA must be greater than or equal to 45% and the lower bound of the 95% two-sided confidence interval must be greater than or equal to 40%.
- With regards to the ColoSense specificity for subjects with negative findings, the specificity must be greater than or equal to 80%.

Secondary Endpoints

The performance characteristics (sensitivity and specificity) of ColoSense were analyzed according to various colonoscopy subgroups for colorectal cancer (CRC), advanced adenomas (AA), and serrated precancerous lesions (SPL). There were no pre-specified acceptance criteria for subgroups.

B. Accountability of PMA Cohort

At the time of database lock, a total of 14,263 subjects were enrolled in the CRC-PREVENT clinical study. A total of 5,974 subjects were excluded from the primary analysis due to:

- returning an invalid ColoSense Collection Kit (e.g., no kit returned or invalid sample),
- not completing a viable colonoscopy (e.g., did not complete colonoscopy or insufficient colonoscopy),
- not completing a colonoscopy within 120 days of collecting a stool sample,
- having invalid medical records (e.g., no medical records received or incomplete medical records),
- having exclusionary criteria on medical records, or
- voluntarily withdrawing from the study.

In total, 8,289 subjects completed all study requirements and had a viable ColoSense test and a viable colonoscopy. Of the 8289 subjects who completed study requirements, there were 526 subjects that had self-reported first-degree relatives with CRC. As such, these individuals were not included in the primary effectiveness population. Therefore, the final average risk cohort consisted of 7,763 subjects who did not have a self-reported first degree relative with CRC and was used for the primary analysis to evaluate the performance of the test in subjects considered typical average risk for CRC as the primary effectiveness cohort.

C. Study Population Demographics and Baseline Parameters

Demographic and baseline risk categories were collected for all subjects. The baseline demographic characteristics for the primary effectiveness cohort are presented in **Table 12**.

The 7,763 subjects in the Average-risk Cohort were derived from all 48 lower U.S. states across more than 5,100 different zip codes. Average population age was 55.3 years (range = 45 years to 90 years) and 59.8% were female (40.1% were male). Regarding race, 80.7% were White, 10.6% were Black or African American, 4.0% were Asian, and 4.7% were other, including American Indian, Alaskan Native, Native Hawaiian, or Pacific Islander. Regarding ethnicity, 6.4%

were Hispanic or Latino. Additionally, 33.5% of subjects had prior tobacco exposure.

Table 12. Demographic Distribution for the Primary Effectiveness Cohort

Characteristic	Average-risk Cohort (n = 7,763)	Average-risk CRC (n = 27)	Average-risk AA (n = 514)	Average-risk SPL (n = 98)	Average-risk NAPL (n = 2,362)	Average-risk NEG (n = 4,762)
Age (range), years	55.3 (45 to 90 years)	55.7 (45 to 74 years)	56.9 (45 to 90 years)	54.4 (45 to 73 years)	56.1 (45 to 83 years)	54.7 (45 to 83 years)
Sex						
Female	4,643 (59.8 %)	17 (63.0 %)	235 (45.7 %)	66 (67.3 %)	1,277 (54.1 %)	3,048 (64.0 %)
Male	3,111 (40.1 %)	10 (37.0 %)	277 (53.9 %)	31 (31.6 %)	1,083 (45.9 %)	1,710 (35.9 %)
Other	9 (0.1 %)	0 (0.0 %)	2 (0.4 %)	1 (1.0 %)	2 (0.1 %)	4 (0.1 %)
Ethnicity						
Hispanic or Latino	500 (6.4 %)	0 (0.0 %)	32 (6.2 %)	5 (5.1 %)	150 (6.4 %)	313 (6.6 %)
Not Hispanic or Latino	7,121 (91.7 %)	26 (96.3 %)	470 (91.4 %)	92 (93.9 %)	2,167 (91.7 %)	4,366 (91.7 %)
No answer	142 (1.8 %)	1 (3.7 %)	12 (2.3 %)	1 (1.0 %)	45 (1.9 %)	83 (1.7 %)
Race						
AI/AN[a]	64 (0.8 %)	0 (0.0 %)	7 (1.4 %)	2 (2.0 %)	24 (1.0 %)	31 (0.7 %)
Asian	307 (4.0 %)	0 (0.0 %)	9 (1.8 %)	3 (3.1 %)	95 (4.0 %)	200 (4.2 %)
Black / African American	821 (10.6 %)	4 (4.8 %)	66 (12.8 %)	4 (4.1 %)	212 (9.0 %)	535 (11.2 %)
NH/PI[b]	17 (0.2 %)	0 (0.0 %)	1 (0.2 %)	0 (0.0 %)	3 (0.1 %)	13 (0.3 %)
White	6,268 (80.7 %)	23 (85.2 %)	409 (79.6 %)	86 (87.8 %)	1,947 (82.4 %)	3,803 (79.9 %)
Other	241 (3.1 %)	0 (0.0 %)	16 (3.1 %)	1 (1.0 %)	71 (3.0 %)	153 (3.2 %)
No answer	45 (0.6 %)	0 (0.0 %)	6 (1.2 %)	2 (2.0 %)	10 (0.4 %)	27 (0.6 %)
Tobacco exposure						
Yes	2,597 (33.5 %)	10 (37.0 %)	256 (49.8 %)	37 (37.8 %)	831 (35.2 %)	1,463 (30.7 %)
No	5,166 (66.5 %)	17 (63.0 %)	258 (50.2 %)	61 (62.2 %)	1,531 (64.8 %)	3,299 (69.3 %)
FH of CRC[c]						
Yes	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
No	7,763 (100.0 %)	27 (100.0 %)	514 (100.0 %)	98 (100.0 %)	2,362 (100.0 %)	4,762 (100.0 %)

[a] American Indian or Alaska Native, [b] Native Hawaiian or Pacific Islander, [c] First-degree relative (parent, sibling, child).

D. Safety and Effectiveness Results

1. Safety Results

Adverse effects that occurred in the PMA clinical study:

Due to the design of the study and nature of the stool collection process, Serious Adverse Events (SAEs) and Serious Adverse Device Effects (SADEs) caused by or related to the stool collection procedure were not anticipated. AE and/or ADEs associated with potential errors in use of the ColoSense Collection Kit and

any product complaints were captured in the safety analyses. Out of 14,263 study participants enrolled, only one (1) ADE was reported during the study. This ADE was considered by the Principal Investigator (PI) to be non-serious and was anticipated. There were no cases in which the PI believed the product contributed to a SAE or a SADE.

The ColoSense test has the related risk of a false test result (i.e., a false positive or a false negative result). All positive test results should lead to a colonoscopy. False positive ColoSense results could lead to an increased number of colonoscopies and associated adverse events related to the colonoscopy procedure. A false negative ColoSense result could lead to a situation where a colorectal cancer, advanced adenoma, or serrated precancerous lesion remains undetected.

2. Effectiveness Results

The analysis of effectiveness was based on the average risk cohort which consisted of 7,763 subjects considered to be at typical average risk for CRC. Key effectiveness outcomes are presented in tables provided in primary effectiveness evaluation below.

a. Primary Effectiveness Evaluation

Cross-tabulation of the ColoSense results by colonoscopy results for the Primary Effectiveness Cohort that includes individuals without a reported first-degree family history of CRC, by clinical category, is provided in **Table 13**.

Table 13. ColoSense outcome for the primary effectiveness cohort by clinical category

ColoSense Result	Histopathological Lesion Category Grouping				Total
	Colorectal cancer (CRC)	Advanced adenomas (AA)	Serrated precancerous lesions (SPL)	All other findings (NEG)	
Positive	25	231	22	1,027	1,305
Negative	2	283	76	6,097	6,458
Total	27	514	98	7,124	7,763

Primary performance characteristics (sensitivity, specificity, positive predictive value, and negative predictive value) for the primary effectiveness cohort, are provided in **Tables 14-15**. Sensitivity for CRC was 92.6%, sensitivity for

advanced precancerous lesions (AA or SPL) was 41.3% and specificity for NAPL or NEG was 85.6%.

Table 14. Sensitivity for colorectal cancer (CRC), advanced precancerous lesions (APL), advanced adenomas (AA), and serrated precancerous lesions (SPL) for the primary effectiveness cohort (7.763 subjects) with associated 95% confidence intervals.

Sensitivity	Category	Ratio	Point Estimate	95% CI
CRC sensitivity	**1.0**	**25 / 27**	**92.6%**	**76% - 99%**
APL (AA and SPL) sensitivity	**2.1-2.4**	**253/612**	**41.3%**	**37% - 45%**
AA sensitivity	2.1-2.3	231 / 514	44.9%	41% - 49%
SPL sensitivity	2.4	22 / 98	22.4%	15% - 32%

Table 15. Specificity for negative findings for the primary effectiveness cohort with associated 95% confidence intervals.

Specificity	Category	Ratio	Point Estimate	95% CI
NAPL or NEG	3.1-6.2	6,097 / 7,124	85.6%	85% - 86%

b. <u>Secondary Effectiveness Evaluation</u>

The ColoSense sensitivity and specificity for various colonoscopy subgroups in the primary effectiveness cohort (i.e., average risk cohort) were analyzed and are presented below. There were no pre-specified acceptance criteria for subgroup analyses.

Table 16 shows secondary performance characteristics by cancer stage and histopathology category. Sensitivity for Stage I CRC was 100.0%, sensitivity for high-grade dysplasia or >10 adenomas was 64.1%, and sensitivity for tubulovillous adenomas was 47.4%.

Table 16. Secondary performance characteristics by colonoscopy categories - sensitivity

Positive Findings	Sensitivity % (+/N)
Colorectal Cancer (CRC) - All	92.6% (25 / 27)
• Stage 1	100.0% (12 / 12)

Positive Findings	Sensitivity % (+/N)
• Stage II	71.4% (5 / 7)
• Stage III	100.0% (8 / 8)
Advanced Adenoma (AA) - All	44.9% (231 / 514)
• High-grade dysplasia or ≥10 adenoma	64.1% (25 / 39)
• Tubulovillous adenoma, any size	47.4% (65 / 137)
• Tubular adenoma, ≥10mm	41.7% (141 / 338)
Serrated Precancerous Lesions (SPL)	22.4% (22 / 98)

Table 17 shows secondary performance characteristics for ColoSense specificity in the clinical effectiveness population. Specificity for NAPL was 83.4% (with 95% CI 82% - 85%), and specificity for NEG was 86.7% (with 95% CI 86% - 88%).

Table 17: Secondary performance characteristics by colonoscopy categories - specificity

Negative Findings	Specificity % (-/N)
NAPL (category 3-5)	83.4% (1969/2362)
NEG findings (category 6)	86.7% (4128/4762)
• Hyperplastic polyps or negative lesions	84.8% (1237/1459)
• No lesions on colonoscopy	87.5% (2891/3303)

Using clinical performance data, three positive predictive values (PPV) and one negative predictive value (NPV) were defined:

- PPV (CRC) = probability of having colorectal cancer given a positive ColoSense result
- PPV (CRC or AA) = probability of having colorectal cancer or advanced adenoma given a positive ColoSense result
- PPV (CRC, AA, or SPL) = probability of having colorectal cancer, advanced adenoma, or serrated precancerous lesion given a positive ColoSense result
- NPV (NAPL or NEG) = probability of having a non-advanced precancerous lesion or a negative finding on colonoscopy given a negative ColoSense result

PPV and NPV as well as 95% confidence intervals for the various cohorts are shown in **Table 18**.

Table 18. Positive predictive value (PPV) and negative predictive value (NPV) for the primary effectiveness cohort with associated 95% confidence intervals.

	Ratio	Point Estimate	95% CI
PPV (CRC)	25 / 1,305	1.9%	1% - 3%
PPV (CRC or AA)	256 / 1,305	19.6%	17% - 22%
PPV (CRC, SPL, or AA)	278 / 1,305	21.3%	19% - 24%
NPV (NAPL or NEG)	6,097 / 6,458	94.4%	94% - 95%

3. Subgroup Analyses

The following baseline characteristics in the primary effectiveness cohort were evaluated for potential association with safety and effectiveness outcomes: age, sex, ethnicity, and race. There were no pre-specified acceptance criteria for subgroup analyses and the study was not specifically powered for these safety and effectiveness outcomes (**Table 19)**.

Table 19. Performance characteristics by demographic factors for the clinical effectiveness population

Demographic Factor	Sensitivity % (+/N)			Specificity % (-/N)
	CRC (n = 27)	AA (n = 514)	SPL (n = 98)	NEG or NAPL (n=7,124)
Age, years				
45-49	5 / 5 (100.0%)	37 / 84 (44.0%)	3 / 26 (11.5%)	1498 / 1705 (87.9 %)
50-54	11 / 11 (100.0%)	68 / 158 (43.0%)	7 / 31 (22.6%)	2070 / 2398 (86.3 %)
55-59	1 / 1 (100.0%)	37 / 82 (45.1%)	5 / 15 (33.3%)	739 / 878 (84.2 %)
60-64	6 / 7 (85.7%)	38 / 88 (43.2%)	5 / 17 (29.4%)	931 / 1119 (83.2 %)
≥65	2 / 3 (66.7%)	51 / 102 (50.0%)	2 / 9 (22.2%)	859 / 1024 (83.9 %)
Sex				
Female	15 / 17 (88.2%)	93 / 235 (39.6%)	12 / 66 (18.2%)	3732 / 4325 (86.3 %)
Male	10 / 10 (100.0%)	137 / 277 (49.5%)	9 / 31 (29.0%)	2360 / 2793 (84.5 %)
Other	-	1 / 2 (50.0%)	1 / 1 (100.0%)	5 / 6 (83.3 %)

Demographic Factor	Sensitivity % (+/N)			Specificity % (-/N)
	CRC (n = 27)	AA (n = 514)	SPL (n = 98)	NEG or NAPL (n=7,124)
Ethnicity				
Hispanic / Latino	-	14 / 32 (43.8%)	0 / 5 (0.0%)	390 / 463 (84.2 %)
Non-hispanic / Latino	25 / 26 (96.2%)	208 / 470 (44.3%)	22 / 92 (23.9%)	5598 / 6533 (85.7 %)
Other	0 / 1 (0.0%)	9 / 12 (75.0%)	0 / 1 (0.0%)	109 / 128 (85.2 %)
Race				
AI/AN[a]	-	4 / 7 (57.1%)	1 / 2 (50.0%)	51 / 55 (92.7 %)
Asian	-	4 / 9 (44.4%)	0 / 3 (0.0%)	264 / 295 (89.5 %)
Black	3 / 4 (75.0%)	30 / 66 (45.5%)	2 / 4 (50.0%)	609 / 747 (81.5 %)
NH/PI[b]	-	0 / 1 (0.0%)	-	14 / 16 (87.5 %)
White	22 / 23 (95.7%)	186 / 409 (45.5%)	19 / 86 (22.1%)	4935 / 5750 (85.8 %)
Other	-	3 / 16 (18.8%)	0 / 1 (0.0%)	188 / 224 (83.9 %)
No answer	-	4 / 6 (66.7%)	0 / 2 (0.0%)	36 / 37 (97.3 %)

[a] American Indian or Alaska Native, [b] Native Hawaiian or Pacific Islander

ColoSense additionally demonstrated benefit over FIT, identifying 4 of 6 (67%) CRC cases that were not identified by FIT. Similarly, for AA detection, ColoSense identified 52 of 335 (16%) AA cases that were not identified by FIT. For SPL detection, ColoSense identified 12 of 88 (14%) SPL cases that were not identified by FIT. Overall sensitivity for CRC, AA and SPL compared to FIT results that are a part of ColoSense test are presented in **Table 20**.

Table 20. Overall sensitivity for ColoSense compared to FIT alone in the clinical effectiveness population

	ColoSense	FIT [b]	Difference
CRC Sensitivity: N = 27 (95% confidence interval) [a]	92.6% (76%-99%)	77.8% (58%-91%)	14.8%
AA Sensitivity: N = 514 (95% confidence interval) [a]	44.9% (41%-49%)	34.8% (31%-39%)	10.1%
SPL Sensitivity: N = 98 (95% confidence interval) [a]	22.4% (15%-32%)	10.2% (5%-18%)	12.2%

[a] Test-statistic confidence intervals were assessed using a two-sided 95% confidence interval
[b] Modified FIT IVD

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal prospective decentralized clinical study included seven (7) investigators of one (1) were full-time or part-time employees of the sponsor and one (1) had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: one
- Significant payment of other sorts: one
- Proprietary interest in the product tested held by the investigator: one
- Significant equity interest held by investigator in sponsor of covered study: one

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. Statistical analyses were conducted by FDA to determine whether the financial interests/arrangements had any impact on the clinical study outcome. The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Having a family history of CRC was not listed as exclusionary criteria for enrollment in the CRC-PREVENT clinical trial. As a consequence, there were 526 subjects who were enrolled as part of the CRC-PREVENT clinical study who reported a first-degree family history of CRC. As such, these individuals were not included in the primary effectiveness population and were analyzed as a separate subgroup. This subgroup analysis was not powered to support a performance for individuals that had first-degree relative(s) with CRC.

The average age for this population was 56.4 years, ranging from 45 to 80 years, 58.0% were female and 42.0% were male. Regarding race, 82.9% were White, 9.1% were Black or African American, and 3.4% were Asian. Regarding ethnicity, 6.5% were Hispanic or Latino. This population included 4 CRC patients, 55 subjects with AA and 7 subjects with SPL. ColoSense results by clinical category in these 526 subjects were as follows: 4/4 CRC detected with 95% CI 39.8%-100% (including 1 Stage I and 3 Stage III CRC); 27/55 AA detected with 95% CI 35.4%-62.9% (histopathology categories 2.1-2.3, including 5/7 high-grade dysplasia or $\geq$10 adenomas, 6/14 tubulovillous adenoma, 16 / 34 tubular adenoma, $\geq$ 10mm), and 2/7 SPL detected with 95% CI 3.7%-71.0% (histopathology category 2.4). For negative findings (histopathology categories 3-6) 315/460 were detected, with 140/176 NAPL

(histopathology categories 3-5) and 246/284 NEG detected (histopathology category 6), including 71/91 of hyperplastic polyp or negative lesions, and 175/193 of no lesions on colonoscopy.

XIII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Data from the analytical studies demonstrated acceptable analytical sensitivity, specificity, and precision of ColoSense.

The pivotal clinical study established ColoSense sensitivity for CRC of 92.6% (2-sided 95% CI LB of 76%) and a specificity of 85.6% (2-sided 95% CI LB 85%) in average risk population for colorectal cancer. For advanced precancerous lesions detection, ColoSense achieved sensitivity of 41.3 %. Finally, ColoSense sensitivity for CRC was demonstrated across age groups, racial/ethnic groups, and in both men and women.

ColoSense additionally demonstrated benefit over FIT, identifying 4 of 6 (67%) CRC cases that were not identified by FIT. Similarly, for AA detection, ColoSense successfully identified 52 of 335 (16%) AA cases that were not identified by FIT. For SPL detection, ColoSense successfully identified 12 of 88 (14%) SPL cases that were not identified by FIT.

In conclusion, the pivotal study demonstrated that ColoSense met the primary performance point estimate measures for sensitivity and specificity of the study. However, because of the lower number of CRC cases the acceptance criteria for the lower bound of 2-sided 95% CI did not meet pre-specified 2-sided 95% CI LB of 80%, achieving 2-sided 95% CI LB of 76%. Therefore, Geneoscopy will perform post-approval study to enroll additional 12,500 participants to achieve 33 additional patients with CRC, including 23 with no family history of CRC.

B. Safety Conclusions

ColoSense is in vitro diagnostic (IVD) test that uses a non-invasive stool collection process, and therefore risks associated with the collection of the stool

sample necessary for the ColoSense test are minimal. During the pivotal clinical study of 14,263 study participants, only one (1) adverse event was reported during the study; this event was considered to be non-serious and was anticipated. There were no cases in which the product contributed to a serious adverse event or a serious adverse device effect. With respect to the ColoSense test itself, as with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. The primary risk associated with the ColoSense test is a false positive or false negative result. Since all positive results should lead to a diagnostic colonoscopy, false positive results may lead to patients being referred to colonoscopy unnecessarily. Adverse events commonly associated with colonoscopy include abdominal discomfort and bowel irregularity post-procedure. Rare adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to the sedation resulting in respiratory and/or cardiac events, stroke and death. In the instance of a false negative result on ColoSense, there is a possibility that a case of colorectal cancer, advanced adenoma, or advanced precancerous adenoma could go undetected. Based on the clinical validation data for ColoSense, there is a chance that as many as 24.3% of patients with colorectal cancer may be missed by this test, given the current data available.

C. Benefit-Risk Determination

Colorectal cancer occurs in approximately 150,000 patients in the United States annually, and is associated with about 50,000 deaths annually, despite uptake of CRC screening via colonoscopy, and non-invasive stool-based tests. Detecting CRC early may lead to significant probable benefit to the public health, as localized CRC has a nearly a 90% 5-year survival rate while metastatic CRC has only approximately a 15% 5-year survival rate.

The probable benefits of the ColoSense device are based on data collected in the CRC-PREVENT study, which was a prospective, blinded, cross-sectional, decentralized study to evaluate ColoSense, with a total of 14,263 participants enrolled in the study. For the original cohort, a total of 5,974 subjects met all study requirement, but were excluded from the primary analysis. Ultimately, the Average Risk cohort consisted of 7,763 subjects who did not have a self-reported first degree relative with CRC and was used for the primary analysis to evaluate the performance of the test in subjects considered typical average risk for CRC as the Primary Effectiveness Cohort in this study.

The study conducted demonstrated probable benefit for CRC detection and detection of advanced precancerous lesions (APL), which consisted of advanced adenomas (AA) and serrated precancerous lesions (SPL). The ColoSense test had a CRC sensitivity of 92.6% (25/27, 2-sided 95% CI: 76-99%) and APL (AA/SPL) sensitivity of 41.3% (253/612). The AA sensitivity was 44.9% (231/514) and SPL sensitivity was 22.4% (22/98). The specificity in the non-NAPL or NEG group was 85.6% (2-sided 95% CI: 85%-86%). The PPV of a finding considered

to be positive (CRC, AA, or SPL) was 21.3% (278/1,305, 2-sided 95% CI: 19-24%), and the NPV in the NAPL or NEG subgroup was 94.4% (6,097-6,458, 2-sided 85% CI: 94-95%).

To take a deeper look, the performance for subgroups was also examined. The sensitivity for Stage I, II and III CRC were 100% (12/12), 71.4% (5/7) and 100% (8/8), respectively, demonstrating probable benefit in detection of early-stage CRC. The sensitivity for high grade dysplasia or ≥ 10 adenomas was 64.1% (25/39), tubulovillous adenomas (any size) was 47.4% (65/137) and serrated precancerous lesions was 22.4% (22/98). Given the totality of the data provided for in the study, the ColoSense device is deemed to have significant probable benefit in the detection of CRC, APL (AA or SPL), with an acceptable level of specificity. Additional probable benefits of this test, include that it is non-invasive and has the potential to detect CRC/APL (AA/SPL) lesions earlier, than without screening, which may translate to better outcomes for patients. Despite the data provided, the probability and magnitude of the benefit of the device for the individual patient may be variable, considering the performance of the device for the patient's condition. However, due to the small number of CRCs in this study (27), there is a chance that as many as 24.3%% of CRC cases could be missed by this device, which represents a major source of uncertainty regarding device performance. Of note, there are already stool based tests for CRC/APL detections approved by the FDA; the availability of this test, provides patients with another option in the screening for CRC/APL, that may have added value over FIT in the detection of CRC.

The probable risks associated with the use of this device, are mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the health care provider. There is minimal probable risk with the collection of stool for the use of this device, since it is non-invasive. When used for screening, a positive result should be followed by colonoscopy for diagnosis. A false positive result could result in an additional invasive screening procedure, such as colonoscopy, and thus unnecessarily expose patients to the attendant risks associated with such a procedure. Rare serious adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to sedation. A false negative result with ColoSense could potentially delay colonoscopy and delay diagnosis of disease such as colorectal cancer, advanced adenoma or serrated precancerous lesions. The consequences of false negatives could be quite serious, such as progression of disease, such as CRC to a more advanced, and less treatable stage. Based on the data provided thus far, there is a chance of up to a 24.3% probability that this test may miss CRC, though the point estimate for CRC sensitivity performance is 92.6% (25/27). To address this risk, the following Warnings and Precautions have been added to the Clinician Brochure:

ColoSense may produce false negative or false positive results. Based on the clinical validation data for ColoSense, with 25/27 colorectal cancers from typical average risk patients identified (92.6%) and with a 95% two-sided confidence

interval lower bound of 75.7%, there is a chance that as many as 24.3% of patients with colorectal cancer may be missed by this test:

- A negative test result does not guarantee the absence of colorectal cancer or precancerous lesions. A false negative (incorrect) result occurs when ColoSense does not detect a colorectal cancer or precancerous lesion even though a colonoscopy could find colorectal cancer or precancerous lesions.
- Positive test result is recommended to be followed up by a colonoscopy. A false positive (incorrect) result occurs when ColoSense produces a positive result even though a colonoscopy will not find colorectal cancer or precancerous lesion.

Due to the uncertainty regarding the CRC sensitivity of this device, additional investigation in the form of post-approval studies are required to confirm the performance of this device for CRC detection, thus far observed.
Additional risks include misinterpretation of results of this test by the health care provider. Despite the mitigations of the labeling, there is residual probable risk that the clinician may not fully understand what a positive or negative result from this test means clinically. This risk has been addressed by provided clear summative tables on device performance in the Clinician Brochure.

Additional factors to be considered in determining probable risks and benefits for the ColoSense included data from rigorous analytical studies, which demonstrated acceptable analytical performance of the test.

1. Patient Perspective

This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that for the qualitative detection of colorectal advanced neoplasia associated with ColoSense RNA markers and for the presence of occult hemoglobin in human stool, the probable benefits of this device outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the CRC-PREVENT clinical trial support the effectiveness of ColoSense to screen for the presence of colorectal cancer, advanced adenoma or serrated precancerous lesions in adults of either sex, 45 years or older, who are average-risk for CRC.

XV. CDRH DECISION

CDRH issued an approval order on May 3, 2024.

The final clinical conditions of approval are cited in the approval order and described in Section XVI below.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

The sponsor will conduct a post-approval study (PAS). The detail of the proposed study are as follows:

Geneoscopy Inc. must conduct a study to expand the total number of colorectal cancer (CRC) cases evaluated by ColoSense. ColoSense Post-Approval Study (PAS), Protocol ID: CT-PRT-0004 is a prospective, multisite, decentralized study evaluating the sensitivity and specificity of the ColoSense screening test for colorectal cancer, using colonoscopy as the reference method. Lesions will be confirmed as malignant by histopathologic examination.

- **Study Objective:** The primary objective of this post-approval study (PAS) is to continue to evaluate the clinical effectiveness of ColoSense by obtaining at least 23 CRC cases (23 cases in addition to the 27 obtained in CRC-PREVENT for a total of 50 CRC cases) to have an adequate number of CRC subjects that are part of the intended use population (average risk) to provide more certainty in the test performance.
- **Sample size:** In total, this study will enroll 12,500 subjects. The ColoSense test will be performed in advance of an average risk screening colonoscopy. With an estimate that 65% of enrolled patients would complete all study requirements, Geneoscopy anticipates approximately 8,125 viable results. Geneoscopy expects to obtain an additional 33 patients with CRC. Of the 33 subjects with CRC, 23 are expected to have no family history of CRC.
- **Study Duration:** The study length will be 36 months with 24 months of follow-up.
    - First subject will be enrolled within 12 months of the study protocol approval date.
    - 20% of subjects enrolled within 20 months of the study protocol approval date.

- 50% of subjects enrolled within 28 months of the study protocol approval date.
- 100% of subjects enrolled within 36 months of the study protocol approval date.

- **Study Endpoints:** Data from the original CRC-PREVENT trial will be pooled with the post-approval study to assess primary endpoints. The primary endpoints for this study are ColoSense sensitivity for colorectal cancer, ColoSense sensitivity for advanced adenomas, ColoSense sensitivity for serrated precancerous lesions, and ColoSense specificity for all other findings. Criteria for success requires that the combined sensitivity of CRC (CRCs observed in the CRC-PREVENT clinical trial and CRCs observed in the PAS) must be greater than 90% and the lower bound of the 95% two-sided confidence interval must be greater than 80% when the data is pooled with the results of the post-approval study.
- The post-approval study will include four co-primary performance measures:
  - ColoSense sensitivity for subjects with Colorectal Cancer (CRC) (category 1.0)
  - ColoSense sensitivity for subjects with Advanced Adenomas (AA) (categories 2.1-2.3)
  - ColoSense sensitivity for serrated precancerous lesions (SPL) (category 2.4) and
  - ColoSense specificity for subjects with negative findings (categories 3.1-6.2)

  The post-approval study will include three co-secondary performance measures:
  - ColoSense sensitivity for subjects with high-grade dysplasia or >10 adenomas (category 2.1)
  - ColoSense sensitivity for subjects with tubulovillous adenomas (category 2.2)
  - ColoSense sensitivity for subjects with advanced adenomas (AA) and serrated precancerous lesions (SPL) (categories 2.1-2.4).

XVII. REFERENCES

The Surveillance, Epidemiology, and End Results (SEER) Program
https://seer.cancer.gov/statfacts/html/colorect.html