



ColoSense® Clinician Brochure



For In-Vitro Diagnostic Use

Rx Only

Prescription Use Only



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Intended Use / Indications For Use

ColoSense is intended for the qualitative 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 iFOBT Analyzer; bioMérieux EMAG Nucleic Acid Extraction System; and Bio-Rad QXDx 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 typical average-risk for developing CRC. ColoSense is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

For *in Vitro* diagnostic use.

For prescription use only.

Contraindications

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

- Personal history of colorectal cancer, adenomas, or other related cancers
- Positive result on another colorectal cancer screening method within the last six 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

Warnings and Precautions

- Patients should **NOT** provide a sample for the ColoSense test if 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, given the current data available.
 - 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 lesions.
 - A negative test result does not guarantee the absence of colorectal cancer or precancerous lesions. A false negative (incorrect) result may occur 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. Programmatic performance of ColoSense (i.e., benefits and risks with repeated testing over an established period of time) has not been studied.
- Non-inferiority or superiority of ColoSense programmatic 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 samples from subjects with Inflammatory Bowel Disease (IBD), breast cancer, gynecological cancers, prostate cancer, and lipomas.
- 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 (IFU) must be followed carefully for accurate results.
- Fecal samples should be treated as if they are potentially infectious.

ColoSense Overview

ColoSense is a multiple-target stool-RNA (mt-sRNA) test that uses RNA markers and occult hemoglobin in human stool to detect colorectal cancer (CRC), advanced adenomas (AA), and serrated precancerous lesions (SPL), as categorized in **Table 1** below. In a pivotal clinical study, ColoSense demonstrated a sensitivity of 92.6% for detection of CRC. Combined sensitivity for AA and SPL was 41.3%, with subcategory sensitivity of 44.9% for detection of AA and 22.4% for the detection of SPL. Specificity was 85.6% for negative findings (including non-advanced precancerous lesions, hyperplastic polyps, other negative lesions, and no lesions on a colonoscopy). Specificity for solely no lesions on a colonoscopy was 87.5%.

Table 1: Classifying Test Results into Categories Based on Colonoscopy Findings

	Test Result	Category	Colonoscopy Findings
Colorectal Cancer (CRC)	Positive	1.0	Stage I-IV colorectal cancer, any size
Advanced Adenomas (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 polyps or negative lesions
		6.2	No lesions on colonoscopy

ColoSense is designed to analyze patients' stool for the presence of 8 RNA molecular biomarkers (GAPDH, ACY1, AREG, TNFRSF10B, CDH1, EGLN2, KRAS, SMAD4) and hemoglobin, supplemented with patient smoking status, which together may indicate the presence of colorectal cancer (CRC), advanced adenomas (AA), or serrated precancerous lesions (SPL).

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). A patient with a positive result should be referred for a follow-up colonoscopy. A patient with a negative result should continue with regular screening. If no result is obtained, a second stool collection may be requested.

Patient Samples for ColoSense

Patients are not required to undergo bowel preparation or follow dietary or medication restrictions to complete the test. Patients should follow the detailed instructions in the ColoSense Collection Kit IFU, which can be found in the collection kit. The collection kit also comes with a container for collecting stool for RNA testing and a separate swab to collect stool for hemoglobin testing. Both the stool sample and the stool swab are required to obtain a ColoSense result. Samples are sent to Geneoscopy's CAP/CLIA accredited laboratory for processing and testing.

ColoSense Patient Services

ColoSense provides patient services to encourage collection kit return from the patient's home to the laboratory. Patient services include live representatives from the ColoSense Customer Service Team available to answer questions about the collection kit, to address billing and reimbursement inquiries, and to send reminders to patients about completing the ColoSense Collection Kit. Please call **1-314-887-7778** or visit www.colosense.com to learn more.

Colorectal Cancer Overview

Colorectal cancer (CRC) is the second leading cause of death from cancers affecting both men and women in the United States. Despite a rise in colorectal cancer (CRC) incidence, particularly among those under 50 years old, CRC screening compliance has remained stagnant at approximately 60% for over 20 years.¹ As a result, over 63% of newly diagnosed patients have advanced disease, with an associated five-year survival rate as low as 12%.² This paradigm has made CRC the second deadliest cancer in the US, causing over 50,000 deaths each year, which highlights the potential benefit of CRC screening.³ While detecting early-stage CRC is paramount for treating and curing disease, detecting precancerous lesions, particularly large adenomatous polyps or adenomas with concerning pathological features (e.g., high-grade dysplasia or villous or tubulovillous architecture), offers patients the highest potential for cancer prevention.⁴ Refer to **Table 1** for information on the detection of adenomas based on size and histology. In 2021, the US Preventive Services Task Force (USPSTF) provided an updated CRC screening recommendation starting at age 45.⁵

Device Description

ColoSense is an in vitro diagnostic (IVD) device designed for the qualitative detection of colorectal neoplasia associated RNA markers and for the presence of occult hemoglobin in human stool. ColoSense evaluates eight (8) RNA markers and hemoglobin concentration. Results from these markers is used in conjunction with smoking status to assess if the patient is likely to have colorectal cancer (CRC), advanced adenomas (AA), or serrated precancerous lesions (SPL).

Completing a ColoSense test requires three steps. First, the ColoSense Collection Kit is sent to the patient's home once a test is prescribed by a Healthcare Provider (HCP). Second, the patient deposits a stool sample into the sample container, and then ships the ColoSense Collection Kit back to Geneoscopy's laboratory. Finally, the stool sample is processed in the Geneoscopy laboratory using the ColoSense Test Kit to generate a ColoSense result. ColoSense involves stool-based testing, which detects biomarkers contained in the cells shed by individuals with CRC, AA, or SPL. The ColoSense output is an aggregated quantification of testing results for RNA biomarkers, FIT/iFOBT, and smoking status.

The ColoSense software is used to assess the various biomarkers and to generate a score, which based on a predefined threshold, yields a positive or negative result for the Healthcare Provider. If the composite score is greater than or equal to the predetermined threshold, the ColoSense result will be reported as positive (abnormal). The patient report sent to the Healthcare Provider will display the result as positive (abnormal) and the patient should be referred for a colonoscopy by the HCP. If the score is below a predetermined threshold, then the result is negative (normal), and the patient will be considered eligible for continued colorectal cancer screening.

Clinical Study: The ColoRectal Cancer and PRE-Cancerous Adenoma Non-Invasive DetectionN Test (CRC-PREVENT)⁶

Overview

The primary objective of the study (CRC-PREVENT) was to determine sensitivity and specificity for the multi-target stool RNA test (ColoSense) using colonoscopy as the reference method. Sensitivity was determined for colorectal cancer, advanced adenomas of select size and number, and serrated precancerous lesions, based on the test correctly calling these findings as “positive”. Specificity was determined based on the test correctly calling all other histological findings as “negative” (see [Table 1](#) for histopathology categories). Lesions were confirmed by histopathologic examination.

Study Design

The CRC-PREVENT clinical trial was a prospective clinical investigation conducted by Geneoscopy. to evaluate ColoSense, which is a multi-target stool RNA test for colorectal neoplasia screening. Subjects were eligible for this study if they were asymptomatic, typical average-risk individuals aged 45 years and older. The study exclusion criteria did not list a family history of CRC.

ColoSense Collection Kits were sent to all subjects who were enrolled in the study. The ColoSense Collection Kit provided all the information and material necessary to collect a stool sample. After a stool sample was produced and collected in the stool container, subjects swabbed the stool sample using the OC-Auto[®] Sampling Bottle according to the IFU and returned both stool samples to Geneoscopy’s laboratory.

Study subjects were directed to receive a colonoscopy within 120 days after providing a stool sample. If a lesion was removed during the colonoscopy, the subject’s tissue sample was sent for histopathology review, as per standard of care practice. A histopathology review was used to determine the index lesion category ([Table 1](#)). The colonoscopy report, histopathology report, physician reports, and pre-operative appointment records were provided to a Clinical Research Organization (CRO) that was blinded to ColoSense results.

Study Populations

In total, 8,289 subjects completed all study requirements within study inclusion / exclusion criteria. Within these 8,289 subjects, there were 526 subjects that had self-reported first-degree relative(s) with CRC. As such, these individuals are not included in the primary effectiveness population (Average-risk Cohort) which consisted of 7,763 subjects at average-risk for CRC. Primary endpoints were assessed for the Average-risk Cohort in the CRC-PREVENT clinical trial. The 526 subjects that had first-degree relative(s) with CRC were analyzed as a separate subgroup (Non-Average-risk Cohort), and results from these subjects are excluded from the primary performance analysis.

Clinical Validation of ColoSense

Clinical Performance Measures and Related Endpoints

The primary and secondary objectives of this study were to determine the sensitivity and specificity of ColoSense using colonoscopy as the reference method. Lesion categories were confirmed by histopathologic examination. The performance of ColoSense was evaluated based on a comparison of the ColoSense result with the histopathological category (see **Table 1**).

The primary objective of the study was assessed using three co-primary performance metrics:

- 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 ColoSense.
- Sensitivity for subjects with advanced adenomas (AA) and serrated precancerous lesions (SPL), which is the percentage of individuals with a diagnosis of advanced adenoma or serrated precancerous lesion (**Table 1**) that were detected as positive by ColoSense.
- Specificity for subjects with Non-advanced Precancerous Lesions (NAPL) or Negative Findings (NEG), which is the percentage of individuals without a diagnosis of CRC, AA, or SPL (**Table 1**) that were detected as negative by ColoSense.

Secondary objectives analyzed various subsets of clinical categories. For secondary objectives, sensitivity performance metrics included: sensitivity for various stages of colorectal cancer (CRC) and sensitivity for various subtypes of advanced adenomas (AA). For secondary objectives, specificity performance metrics included: specificity for non-advanced precancerous lesions (NAPL), negative findings (NEG), and no lesions on colonoscopy.

Study Population and Baseline Demographics (Clinical Effectiveness Population)

The baseline demographic characteristics for the Average-risk Cohort (primary effectiveness population) is presented in **Table 2** below.

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. The 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. Across all subjects, 29.0% of patients had an income below \$50,000 per year and 28.3% were on public insurance or had no insurance. Additionally, 33.5% of subjects had prior tobacco exposure.

Table 2: Baseline Demographics for the Average-risk Cohort

Characteristic	Subgroup	Primary Effectiveness Population (n = 7,763)
Age	Mean (range)	55.3 (45 years to 90 years)
	45-49	1,820 (23.4%)
	50-54	2,598 (33.5%)
	55-59	976 (12.6%)
	60-64	1,231 (15.9%)
	>65	1,138 (14.7%)
Sex	Female	4,643 (59.8%)
	Male	3,111 (40.1%)
	Other	9 (0.1%)
Race	American Indian / Alaskan Native	64 (0.8%)
	Asian	307 (4.0%)
	Black / African American	821 (10.6%)
	Native Hawaiian / Other Pacific Islander	17 (0.2%)
	White	6,268 (80.7%)
	Other	286 (3.7%)
Ethnicity	Hispanic or Latino	500 (6.4%)
	Not Hispanic or Latino	7,121 (91.7%)
	Prefer not to Answer	142 (1.8%)
Tobacco Exposure	Yes	2,597 (33.5%)
	No	5,166 (66.5%)
Family History of CRC (first-degree relative)	Yes	0 (0.0%)
	No	7,763 (100%)

Performance Characteristics for the Average-risk Cohort (Clinical Effectiveness Population)

Table 3 shows the primary performance characteristics of ColoSense. Sensitivity for CRC was 92.6%, sensitivity for AA or SPL was 41.3%, and specificity for NAPL or NEG was 85.6%.

Table 3: Primary Performance Characteristics for the Average-risk Cohort

Sensitivity (+/n) [95% CI]	Average-risk Cohort (n = 7,763)
Sensitivity (CRC)	92.6% (25 / 27) [76% - 99%]
Sensitivity (AA or SPL)	41.3% (253 / 612) [37% - 45%]
Specificity (-/n) [95% CI]	Average-risk Cohort (n = 7,763)
Specificity (NAPL or NEG)	85.6% (6,097 / 7,124) [85% - 86%]

Table 4 and **Table 5** show secondary performance characteristics for ColoSense, by histopathology categories and by cancer stage. Sensitivity for AA was 44.9% and sensitivity for SPL was 22.4%. Specificity for NAPL was 83.4%, specificity for NEG was 86.7%, and specificity for no lesions on colonoscopy was 87.5%. 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 4: Secondary Performance Characteristics for the Average-risk Cohort

Sensitivity (+/n) [95% CI]	Average-risk Cohort (n = 7,763)
Sensitivity (AA)	44.9% (231 / 514) [41% - 49%]
Sensitivity (SPL)	22.4% (22 / 98) [15% - 32%]
Specificity (-/n) [95% CI]	Average-risk Cohort (n = 7,763)
Specificity (NAPL)	83.4% (1,969 / 2,362) [82% - 85%]
Specificity (NEG)	86.7% (4,128 / 4,762) [86% - 88%]
Specificity (no lesions on colonoscopy)	87.5% (2,891 / 3,303) [86% - 89%]

Table 5: ColoSense Results by Clinical Category for the Average-risk Cohort

Category	Average-risk Cohort (n = 7,763)
Positive Findings	Sensitivity (+/n) [95% CI]
Colorectal Cancer (CRC)	92.6% (25 / 27) [76%-99%]
Stage I	100.0% (12 / 12) [74%-100%]
Stage II	71.4% (5 / 7) [29%-96%]
Stage III	100.0% (8 / 8) [63%-100%]
Unknown	-
Advanced Adenomas (AA)	44.9% (231 / 514) [41%-49%]
High-grade dysplasia or ≥10 adenomas	64.1% (25 / 39) [47%-79%]
Tubulovillous adenomas, any size	47.4% (65 / 137) [39%-56%]
Tubular adenoma, ≥ 10mm	41.7% (141 / 338) [36%-47%]
Serrated Precancerous Lesions (SPL)	22.4% (22 / 98) [15%-32%]
Negative Findings	Specificity (-/n) [95% CI]
Non-advanced Precancerous Lesions (NAPL)	83.4% (1,969 / 2,362) [82%-85%]
Negative Findings (NEG)	86.7% (4,128 / 4,762) [86%-88%]
Hyperplastic Polyps or negative lesions	84.8% (1,237 / 1,459) [83%-87%]
No lesions on colonoscopy	87.5% (2,891 / 3,303) [86%-89%]

Table 6 shows performance of the ColoSense test by demographic subgroups.

Table 6: Performance by Demographic Subgroup for the Average-risk Cohort

Subgroup	Sensitivity (CRC)	Sensitivity (AA)	Sensitivity (SPL)	Specificity (NAPL)	Specificity (NEG)
Gender					
Female	15 / 17 (88.2%)	93 / 235 (39.6%)	12 / 66 (18.2%)	1,076 / 1,277 (84.3%)	2,656 / 3,048 (87.1%)
Male	10 / 10 (100.0%)	137 / 277 (49.5%)	9 / 31 (29.0%)	891 / 1,083 (82.3%)	1,469 / 1,710 (85.9%)
Other	-	1 / 2 (50.0%)	1 / 1 (100.0%)	2 / 2 (100.0%)	3 / 4 (75.0%)
Age					
45-49	5 / 5 (100.0%)	37 / 84 (44.0%)	3 / 26 (11.5%)	426 / 485 (87.8%)	1,072 / 1,220 (87.9%)
50-54	11 / 11 (100.0%)	68 / 158 (43.0%)	7 / 31 (22.6%)	639 / 767 (83.3%)	1,431 / 1,631 (87.7%)
55-59	1 / 1 (100.0%)	37 / 82 (45.1%)	5 / 15 (33.3%)	249 / 304 (81.9%)	490 / 574 (85.4%)
60-64	6 / 7 (85.7%)	38 / 88 (43.2%)	5 / 17 (29.4%)	303 / 379 (79.9%)	628 / 740 (84.9%)
>65	2 / 3 (66.7%)	51 / 102 (50.0%)	2 / 9 (22.2%)	352 / 427 (82.4%)	507 / 597 (84.9%)
Race					
AI/AN	-	4 / 7 (57.1%)	1 / 2 (50.0%)	21 / 24 (87.5%)	30 / 31 (96.8%)
Asian	-	4 / 9 (44.4%)	0 / 3 (0.0%)	81 / 95 (85.3%)	183 / 200 (91.5%)
Black	3 / 4 (75.0%)	30 / 66 (45.5%)	2 / 4 (50.0%)	173 / 212 (81.6%)	436 / 535 (81.5%)
NH/OPI	-	0 / 1 (0.0%)	-	2 / 3 (66.7%)	12 / 13 (92.3%)
White	22 / 23 (95.7%)	186 / 409 (45.5%)	19 / 86 (22.1%)	1,628 / 1,947 (83.6%)	3,307 / 3,803 (87.0%)
Other	-	3 / 16 (18.8%)	0 / 1 (0.0%)	55 / 71 (77.5%)	133 / 153 (86.9%)
No answer	-	4 / 6 (66.7%)	0 / 2 (0.0%)	9 / 10 (90.0%)	27 / 27 (100.0%)
Ethnicity					
Hispanic or Latino	-	14 / 32 (43.8%)	0 / 5 (0.0%)	123 / 150 (82.0%)	267 / 313 (85.3%)
Not Hispanic or Latino	25 / 26 (96.2%)	208 / 470 (44.3%)	22 / 92 (23.9%)	1,811 / 2,167 (83.6%)	3,787 / 4,366 (86.7%)
Other	0 / 1 (0.0%)	9 / 12 (75.0%)	0 / 1 (0.0%)	35 / 45 (77.8%)	74 / 83 (89.2%)

Using the data above, 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

Table 7 shows the PPV and NPV for the ColoSense test in the intended use population. As with other CRC screening tests, the PPV is impacted by the very low prevalence of CRC in the general population.

Table 7: Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for the Average-risk Cohort

Statistic	Average-risk Cohort (n = 7,763)
PPV (CRC)	1.9% (25 / 1,305) [1% - 3%]
PPV (CRC or AA)	19.6% (256 / 1,305) [17% - 22%]
PPV (CRC, AA, or SPL)	21.3% (278 / 1,305) [19% - 24%]
NPV (NAPL or NEG)	94.4% (6,097 / 6,458) [94% - 95%]

Performance in the Non-Average-risk Cohort

The original clinical study of 8,289 subjects included the Non-Average-risk Cohort consisting of 526 subjects with a first-degree family history of CRC, which were excluded from clinical performance assessments in the average-risk population. Average age for this cohort was 56.4 years (range = 45 years to 80 years) and 58.0% were female (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. Across all subjects, 34.8% of patients had an income below \$50,000 per year and 33.1% were on public insurance or had no insurance. This subgroup analysis was not powered to support a performance for individuals that had first-degree relative(s) with CRC.

This population included 4 CRC patients, 55 subjects with AA, and 7 subjects with SPL. ColoSense results by clinical category in these 526 subjects are shown in **Table 8**.

Table 8: ColoSense Results by Clinical Category for the Non-Average-risk Cohort

Category	Non-Average-risk Cohort (n = 526)
Positive Findings	Sensitivity (+/n) [95% CI]
Colorectal Cancer (CRC)	
Stage I	100.0% (1 / 1) [2% - 100%]
Stage II	100.0% (3 / 3) [30% - 100%]
Stage III	-
Unknown	-
Advanced Adenomas (AA)	
High-grade dysplasia or ≥10 adenomas	71.4% (5 / 7) [29% - 96%]
Tubulovillous adenomas, any size	42.9% (6 / 14) [18% - 71%]
Tubular adenoma, ≥ 10mm	47.1% (16 / 34) [30% - 65%]
Serrated Precancerous Lesions (SPL)	28.6% (2 / 7) [4% - 71%]
Negative Findings	Specificity (-/n) [95% CI]
Non-advanced Precancerous Lesions (NAPL)	79.5% (140 / 176) [73% - 85%]
Negative Findings (NEG)	86.6% (246 / 284) [82% - 90%]
Hyperplastic Polyps or negative lesions	78.0% (71 / 91) [68% - 86%]
No lesions on colonoscopy	90.7% (175 / 193) [86% - 94%]

Cross-Reactivity

The potential for cross-reactivity with non-colorectal cancers and inflammatory conditions was evaluated by testing 131 subjects across 14 different diseases. Subjects with known disease status provided a stool sample, which was assessed with the ColoSense test to generate ColoSense scores. False positive results were extrapolated by disease incidence to assess the impact of disease on ColoSense specificity. The results are shown in **Table 9**.

Table 9: ColoSense Positivity Rates for Non-CRC Diseases and Cancers

Disease	Number of samples tested	Incidence rate per 10,000 ^a	% ColoSense Positive	Projected 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
Prostate Cancer ^d	19	11.27	31.6%	1.8
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

^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.

Interfering Substances

The molecular component of the test was assessed with substances that could potentially be found in patient samples, including common lotions and creams, feminine over-the-counter products, stool softeners, anti-diarrhea products, laxatives, antacids, upset stomach relief products, common edible animal products, urine, alcohol, common vegetables and fruits, and fecal fats. There was no observed interference with any substance tested in the assay.

Ordering ColoSense

ColoSense may be ordered by healthcare providers through electronic ordering systems or paper requisition. Live representatives from the ColoSense Customer Service Team provide attentive service to healthcare providers and their patients. For any questions about ColoSense or specific questions on how to order the test, please contact Geneoscopy.

Geneoscopy, Inc.

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colosense.com

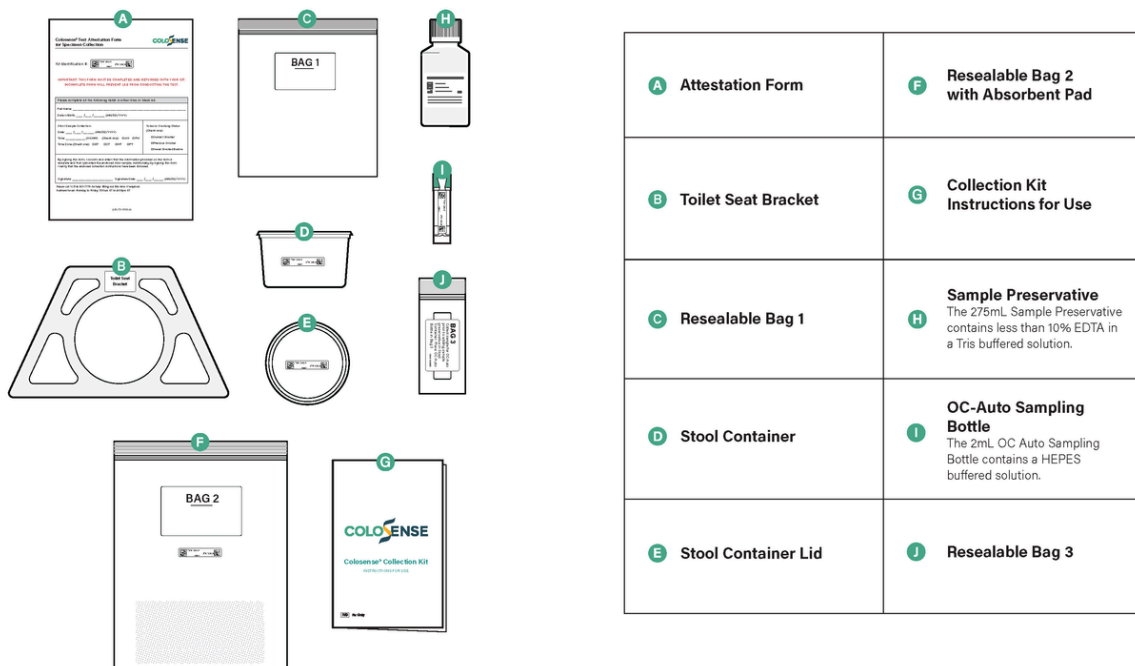
Instructions for Sample Collection

Once the healthcare provider orders ColoSense, the ColoSense Collection Kit will be sent to the patient's residence. Within the kit, the ColoSense Collection Kit IFU will provide detailed instructions for patient sample collection. A phone number to contact the ColoSense Customer Service Team is also provided within the ColoSense Collection Kit to ensure that any patient questions are answered. The below figure provides an overview of the collection process.

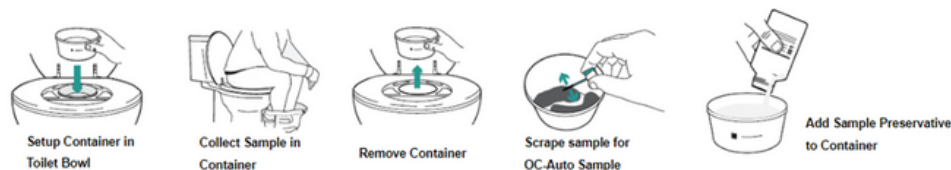
Figure 1. Patient Sample Collection Process

Step 1: Check and prepare contents of the ColoSense Collection Kit

ColoSense Collection Kit Contents



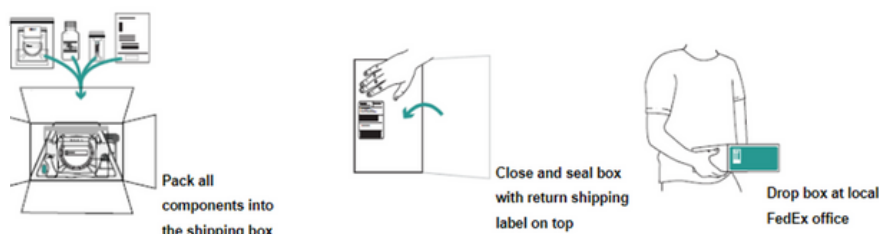
Step 2: Collect, swab, and then add buffer to the stool sample



Step 3: Close and seal the sample and complete the Sample Attestation Form



Step 4: Pack the ColoSense Collection Kit for return shipping, and deliver to a convenient FedEx location



Interpretation of ColoSense Results

Patients are advised to contact their healthcare provider to discuss the ColoSense Result.

The test result can be **Positive**, **Negative**, or **No Result**.

POSITIVE: A positive result means the test detected abnormal RNA expression and/or blood that could be caused by precancerous adenomas/lesions or cancer in the colon or rectum. A test can also have a positive result that is incorrect (false positive). A false positive result means the patient has a positive ColoSense test, but a colonoscopy would not identify precancerous adenomas/lesions or cancer. A diagnostic colonoscopy should follow any positive result.

NEGATIVE: A negative result means the test did not detect abnormal RNA expression or blood in the sample that could be caused by precancerous adenomas/lesions or cancer in the colon or rectum. A test can also have a negative result that is incorrect (false negative). A false negative result means that the patient has a negative ColoSense test, but a colonoscopy would identify precancerous adenomas/lesions or cancer. For that reason, it is important to continue a regular screening schedule with your patients.

NO RESULT: In some cases, ColoSense may not generate a result. A no result means the test was not able to produce a valid result. When this occurs, the patient is advised to consult with their healthcare provider about the next steps for screening, which may include providing another sample.

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6. Barnell EK, Wurtzler EM, La Rocca J, Fitzgerald T, Petrone J, Hao Y, Kang Y, Holmes FL, Lieberman DA. Multitarget Stool RNA Test for Colorectal Cancer Screening. JAMA. 2023 Nov 14;330(18):1760-1768. doi: 10.1001/jama.2023.22231. PMID: 37870871.



For In-Vitro Diagnostic Use

Rx Only Prescription Use Only

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LBL-TD-0010v3



Geneoscopy, Inc.
Saint Louis, MO 63146



ColoSense® Patient Brochure



For In-Vitro Diagnostic Use

Rx Only

Prescription Use Only



 **Geneoscopy, Inc.**
2220 Welsch Industrial Ct.
St. Louis, MO 63146

www.geneoscopy.com
314-887-7777

Intended Use / Indication for Use

ColoSense is intended for the qualitative 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 iFOBT Analyzer; bioMérieux EMAG Nucleic Acid Extraction System; and Bio-Rad QXDx 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 typical average-risk for developing CRC. ColoSense is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

For *in Vitro* diagnostic use.

For prescription use only.

Contraindications

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

- Personal history of colorectal cancer, adenomas, or other related cancers
- Positive result on another colorectal cancer screening method within the last six 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

Warnings and Precautions

- Patients should **NOT** provide a sample for the ColoSense test if 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, given the current data available.
 - 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 lesions.
 - A negative test result does not guarantee the absence of colorectal cancer or precancerous lesions. A false negative (incorrect) result may occur 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 cancer screening (i.e., repeated testing over an established period of time) with ColoSense has not been studied.
- Non-inferiority or superiority of ColoSense programmatic sensitivity as compared to other recommended screening methods for colorectal cancer, advanced adenomas, or serrated precancerous lesions has not been established.
- Patients should be careful when opening or closing lids in the ColoSense Collection Kit to avoid hand strain. Ensure containers are properly sealed prior to shipping.
- Do not drink the bottle of Sample Preservative Buffer and keep away from children. Do not let the liquid touch your skin or eyes. If the liquid touches your skin or eyes, rinse thoroughly with water.
- 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.
- Patients should ensure the attestation form is complete and placed in the ColoSense Collection Kit for return shipment.
- The Instructions for Use must be followed carefully for accurate results.
- Fecal samples should be treated as if they are potentially infectious.

Colorectal Cancer (CRC) Screening

Colorectal cancer is one of the most preventable types of cancer when found early. Unfortunately, many people are not up to date with recommended screening guidelines, which has led colorectal cancer to become the third most common cancer and the second leading cause of cancer-related deaths in both men and women.¹

Colorectal cancer can be detected early if you are looking for it. Colorectal cancer grows slowly, generally starting from small, precancerous polyps in the colon or rectum. A polyp is an abnormal growth in the inner wall of your colon or rectum and is relatively common in people 45 years of age and older. While most polyps are benign, some can be dangerous due to their ability to turn into cancer over time.¹ For this reason, clinical guidelines recommend that average-risk adults undergo colorectal cancer screening beginning at age 45. If colorectal cancer is detected in its early stages through screening, treatment is more likely to be successful.² ColoSense can help you screen for colorectal cancer and precancerous polyps.

ColoSense for CRC Screening

ColoSense works by detecting RNA and hemoglobin in cells that shed from the lining of your colon and accumulate in stool. The ColoSense screening test can detect abnormalities within these cells to determine the presence of colorectal cancer or precancerous polyps.

ColoSense results are binary: **POSITIVE** or **NEGATIVE**.

- If a stool test result is **POSITIVE**, your entire colon should be examined by colonoscopy.
- If your test result is **NEGATIVE**, you should continue with a regular screening schedule.

If **NO RESULT** is obtained, you will be contacted and may be asked to provide another stool sample.

You can also go to www.colosense.com to learn more about ColoSense.

Clinical Study Results - Is ColoSense accurate and effective in finding cancer and precancer?

In a large prospective clinical trial³, ColoSense was studied for its ability to detect colorectal cancer and precancer compared to a colonoscopy. The trial included adults ages 45 and older who were of typical average-risk for developing colorectal cancer. Each person in the study completed the ColoSense test before receiving a screening colonoscopy.

In the clinical trial, 7,763 subjects at average-risk for colorectal cancer successfully completed the ColoSense test and the subsequent colonoscopy. For these patients, ColoSense correctly identified 93% of participants with colorectal cancer and 41% of participants with precancers. The ColoSense test correctly provided a negative screening result for 86% of the trial participants with all other findings / no disease on a colonoscopy. This means that 14% of study subjects without colorectal cancer or precancer tested positive with ColoSense.

Ordering ColoSense and Who to Call with Questions

ColoSense can only be ordered by a healthcare provider (HCP). Once ordered, a ColoSense Collection Kit will be shipped to your home for sample collection. If you have questions, ColoSense has a Customer Service team to answer questions about using and returning the collection kit and provide educational resources on the importance of screening.

To contact ColoSense Customer Service, please call **1-314-887-7778** or visit **www.colosense.com**.

Sample Collection Process

- When you are ready to have a bowel movement, follow the instructions provided in the ColoSense Collection Kit instructions.
- You do not need to undergo any bowel preparation or follow a dietary or medication restriction to complete the test.
- Once you have successfully produced a sample, return the ColoSense Collection Kit using the pre-paid shipping label provided.
- The laboratory will process your sample and generate a ColoSense test result which will be sent to the healthcare provider that ordered your test.
- Your healthcare provider will share your results with you.

Understanding Your ColoSense Result

A **POSITIVE** result means the test detected abnormal RNA and/or blood that could be caused by cancer or precancer in the colon or rectum.

- The test can also have a positive result that is incorrect (false positive). This means the test result is positive, but no cancer or precancer is actually present.
- Any positive result should be followed by a colonoscopy.
- Talk about your test result with your doctor to find out if additional testing is needed.

A **NEGATIVE** result means the test did not detect abnormal RNA or blood in the sample that could be caused by cancer or precancer in the colon or rectum.

- The test can also have a negative result that is incorrect (false negative). A false negative result means that the test result missed a potential cancer or precancer. For that reason, it is important to continue to schedule regular screenings. Your doctor may recommend an alternative screening method.
- Talk to your doctor about your test result. Your provider will recommend a screening schedule that is best for you.

In some cases, a **NO RESULT** may occur if the sample cannot be processed. A no result means the test was not able to produce a valid result. If this occurs, please consult with your doctor about the next steps for screening, which may include providing another sample.

ColoSense May Not be for Everyone

Talk to your doctor if any of the following apply to you:

- Personal history of colorectal cancer, adenomas, or other related cancers
- You had a positive result on another colorectal cancer screening method within the last six months, or completed the following:
 - Fecal occult blood test (FOBT) or fecal immunochemical test (FIT) within the last 12 months
 - FIT-DNA Test within the last 36 months
- You have been diagnosed with any of the following conditions that place you at high-risk 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
- You have been diagnosed with 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

Risks and Precautions Associated with using ColoSense

- **Do 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
- Patients should be careful when opening or closing lids in the ColoSense Collection Kit to avoid hand strain. Ensure containers are properly sealed prior to shipping.
- Do not drink the bottle of Sample Preservative Buffer and keep away from children. Do not let the liquid touch your skin or eyes. If the liquid touches your skin or eyes, rinse thoroughly with water.
- 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.
- Patients should ensure the attestation form is complete and placed in the ColoSense Collection Kit for return shipment.
- The Instructions for Use must be followed carefully for accurate results.
- Fecal samples should be treated as if they are potentially infectious.

Have Questions?

If you have questions, please call our Customer Service team at **314-887-7778**. Additional information is also available online at www.colosense.com.

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ColoSense® Collection Kit

INSTRUCTIONS FOR USE



Rx Only

Intended Use

ColoSense is intended for the qualitative 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 iFOBT Analyzer; bioMérieux EMAG Nucleic Acid Extraction System; and Bio-Rad QXDx 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 adenoma (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 typical average risk for developing CRC. ColoSense is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

To report any adverse events from using this kit, or if you need assistance, please call ColoSense patient services at (314) 887-7778.

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Glossary

Colon: Part of the human digestive system also known as the large intestine.

Colorectal Cancer (CRC): Abnormal growth on the wall of the colon or rectum that is malignant.

Colonoscopy: A medical procedure in which a flexible instrument is inserted through the anus in order to examine the colon.

Hemoglobin: A compound in blood. Blood or hemoglobin in the stool that cannot be seen by the naked eye is referred to as occult blood.

Neoplasia: The abnormal growth. Neoplasia can be a precancerous polyp (benign) or cancerous (malignant).

Precancerous polyps: Abnormal growth on the wall of the colon or rectum that is not cancer but may develop into cancer.

RNA: Ribonucleic acid - RNA is a copy of genetic material in cells. Cells are sloughed off into the stool, releasing RNA into the stool. Analysis of the RNA in stool can provide information about whether cancerous and precancerous cells are present in the colorectum.

Summary of the Test

Screening for colorectal cancer can often find colorectal cancer early, when it's small, hasn't spread, and might be easier to treat. Regular screening can even prevent colorectal cancer by detecting and removing polyps before they have the chance to turn into cancer. A polyp can take as many as 10 to 15 years to develop into cancer.

ColoSense® is a screening test that is designed to use a bowel movement (stool sample) to detect the presence of abnormal RNA and fecal occult blood which may be indicative of colorectal cancer and precancerous polyps.

The ColoSense® Collection Kit is designed to collect your stool sample and preserve it during transport back to the laboratory for testing. After returning your stool sample to the laboratory, the laboratory will complete testing and report the results to your healthcare provider. Your provider will contact you with the test results.

NOTE: No changes to diet or medications are required for this screening test.

Principle of the Procedure

The ColoSense® test combines information about you (Attestation Form) with tests for RNA (stool sample) and blood (fecal swab) to generate a ColoSense® score. The score can tell us if you have colorectal neoplasia, which includes colorectal cancer and precancerous polyps.

Storing your Collection Kit

Store the ColoSense Collection Kit at room temperature, out of the reach of children, and out of direct sunlight until ready for use.

Contraindications

ColoSense is NOT indicated for use for patients who have the following:

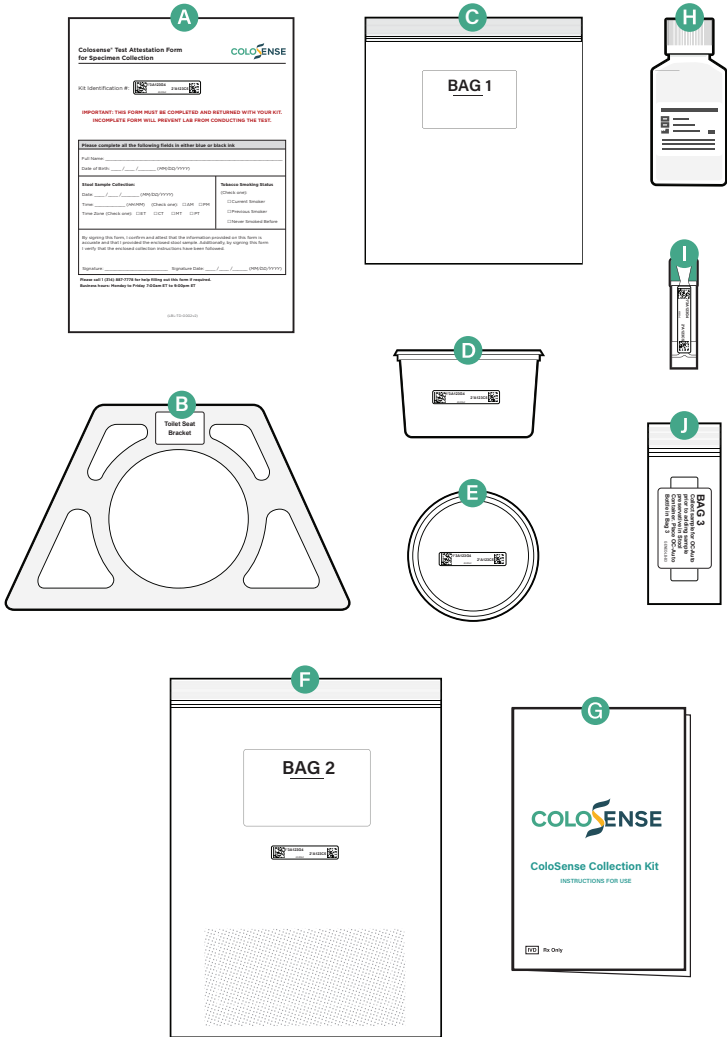
- **Personal history of colorectal cancer, adenomas, or other related cancers.**
- **Positive result on another colorectal cancer screening method within the last six months, or:**
 - ▷ 12 months for fecal occult blood test (FOBT) or fecal immunochemical test (FIT)
 - ▷ 36 months for FIT-DNA test
- **Personal 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
 - ▷ Familial Hyperplastic Polyposis

Before You Start: Warnings and Precautions

Do NOT provide a sample for the ColoSense test if you have the following:

- **Bleeding hemorrhoids**
- **Bleeding cuts or wounds on your 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, given the current data available.
 - A false positive (incorrect) result occurs when ColoSense produces a positive result when colorectal cancer or precancers are not present.
 - A false negative (incorrect) result may occur when ColoSense does not detect colorectal cancer or precancers that are present in the colon or rectum.
- A positive test result indicates that colorectal cancer or precancers may be present in the colon or rectum. It is recommended that positive results be followed up by a colonoscopy.
- A negative test result indicates colorectal cancer or precancers were not detected.
- Benefits and risks with repeated ColoSense testing over an established period of time has not been studied.
- **Do NOT drink the bottle of Sample Preservative and keep away from children.**
- **Do NOT let the liquid touch your skin or eyes.** If the liquid touches your skin or eyes, rinse thoroughly with water.
- **Do NOT apply green top sample probe to sample after pouring on Sample Preservative.**
- **Do NOT mix components from different Collection Kits.**
- Fecal samples should be treated as if they are potentially infectious.
- The Instructions for Use must be followed carefully for accurate results.
- Opening or closing lids in the kit may be difficult for some. Be careful to avoid hand strain and ensure containers are properly sealed prior to shipping.
- Ensure the attestation form is complete and placed in box for return shipment.
- 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.

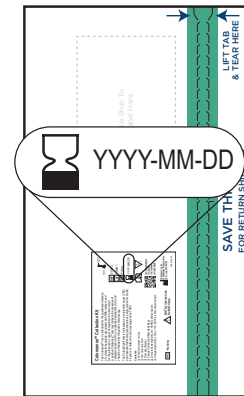
ColoSense Collection Kit Contents



A Attestation Form	F Resealable Bag 2 with Absorbent Pad
B Toilet Seat Bracket	G Collection Kit Instructions for Use
C Resealable Bag 1	H Sample Preservative The 275mL Sample Preservative contains less than 10% EDTA in a Tris buffered solution.
D Stool Container	I OC-Auto Sampling Bottle The 2mL OC Auto Sampling Bottle contains a HEPES buffered solution.
E Stool Container Lid	J Resealable Bag 3

Collecting Your Stool Sample

1.

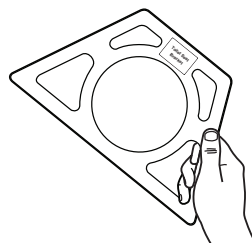


Check the expiration date (🕒 YYYY-MM-DD) on the label on the outside of the Collection Kit to ensure the kit is NOT expired. If the kit is expired, please contact ColoSense patient services at (314) 887-7778.

2.

Samples should be shipped back within 24 hours of collection. **Perform the following steps only if you can ship your sample within the next 24 hours.**

3.



Remove **Toilet Seat Bracket** from box.

6.



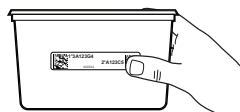
Lower toilet seat down over **Toilet Seat Bracket** making sure the circular opening of bracket is visible.

4.



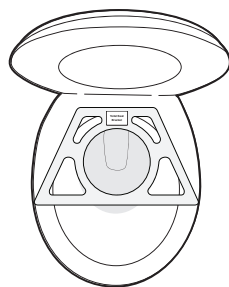
Raise toilet seat and lid.

7.



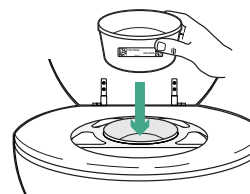
Remove **Stool Container** from box.

5.



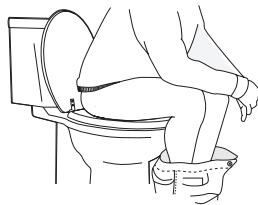
Place **Toilet Seat Bracket** onto the toilet bowl so that it sits on top of the bowl and beneath the seat with the short end towards the back.

8.



Place **Stool Container** into bracket opening.

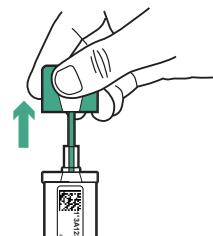
9.



Sit on the toilet and have a bowel movement into the container.

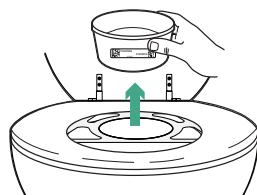
Do NOT collect urine
Do NOT collect bloody, loose, or pelleted stool.

12.



While holding the bottle in an upright position (contains liquid Sample Preservative), pull upwards to open.

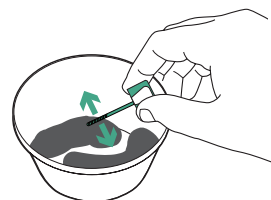
10.



Once finished, lift **Stool Container** from bracket and place on a level, stable surface. The **Toilet Seat Bracket** can be recycled, disposed of, or shipped back in your collection kit.

Do NOT seal Stool Container before swabbing sample and applying Sample Preservative.

13.



Randomly scrape several sections of the stool sample with the sample probe. Ensure the grooved portion is completely covered in stool.

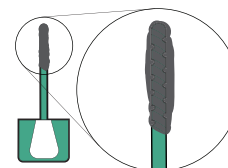
Do NOT scrape stool after addition of Sample Preservative. If Sample Preservative is added prior to using the sample probe, contact ColoSense patient services for a new Collection Kit.

11.

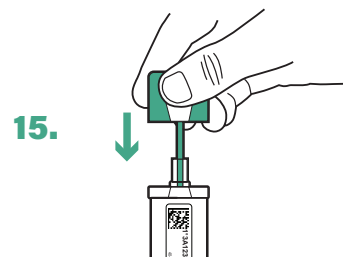


Remove **OC-Auto Sampling Bottle** from box.

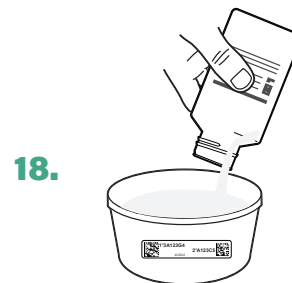
14.



Confirm the grooved portion of the sample probe is completely covered before closing.



Close **OC-Auto Sampling Bottle** by re-inserting the sample probe into the bottle and pressing down until the cap seals on. Shake the bottle gently a few times to mix the stool sample with the Sample Preservative. The bottle is finished and can be set aside for now.



Pour **Sample Preservative** into **Stool Container** to completely cover the stool sample.

Pour the Sample Preservative into the Stool Container. Use the entire bottle or enough to cover the stool sample.



Remove the bottle of **Sample Preservative** from the box.

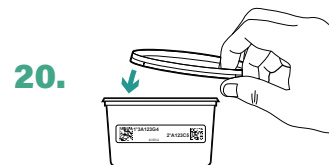
NOT for internal use. Do NOT drink. Do NOT add Sample Preservative before using the OC-Auto Sampling Bottle to scrape the sample.



Return the cap to the top of the **Sample Preservative** and turn the cap clockwise to close. Set bottle back in the box.

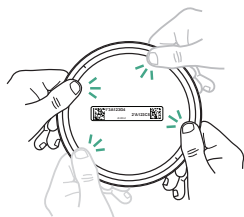


Turn the bottle cap counterclockwise to open.



Place the **Stool Container Lid** on top of the sample container.

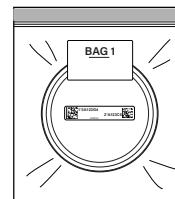
21.



Press down tightly on the **Stool Container Lid** until feeling the container seal. Press down around the edges until you feel the lid seal onto the bucket.

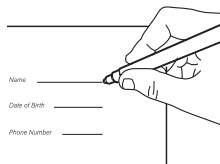
Do NOT pull tabs on side of lid or attempt to reopen container.

24.



Insert **Stool Container** into **Resealable Bag 1**. Remove all air from bag as best as you can and seal bag.

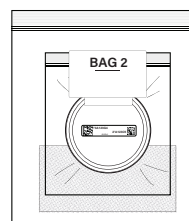
22.



Fill in all required information on the **Attestation Form**.

NOTE: A complete Attestation Form is required to process your sample.

25.



Insert **Bag 1** with the closed **Stool Container** into **Bag 2**. Remove all air from bag as best as you can and seal bag.

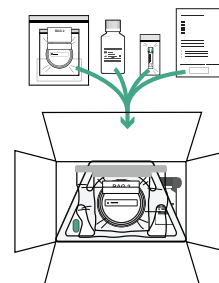
NOTE: Please do not remove Absorbent Pad from Bag 2.

23.



Insert **OC-Auto Sampling Bottle** into **Bag 3**. Remove air as best you can and seal bag.

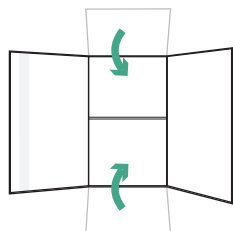
26.



Place each of the following into the shipping box. Any remaining materials can be discarded.

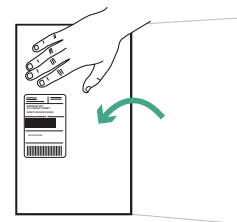
- ☐ **Stool Container in Sealed Bags**
- ☐ **Sample Preservative**
- ☐ **OC-Auto Sample Preservative Bottle in Bag 3**
- ☐ **Completed Attestation Form**

27.



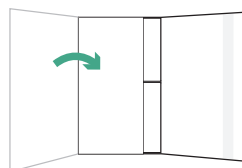
Fold smaller flaps of box over first.

30.



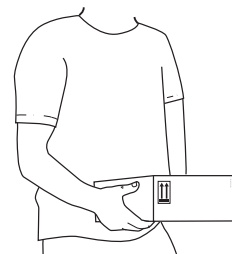
Fold flap with adhesive strip and make sure the FedEx return label is visible on top. Press down to seal.

28.



Fold flap without adhesive strip over the two smaller flaps.

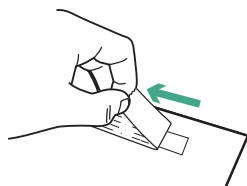
31.



Deliver to nearest FedEx location within 24 hours.

Visit [Fedex.com](https://www.fedex.com) or call 1 (800) 463-3339 to see drop-off locations near you.

29.



Remove adhesive strip from box to expose adhesive.

Shipping Your Stool Sample

Date Collected	Last Date to be Shipped (Before Noon)
Sunday	Monday
Monday	Tuesday
Tuesday	Wednesday
Wednesday	Thursday
Thursday	Friday
Friday*	Friday
Saturday*	Saturday

*FedEx may not pickup or deliver on Saturday or Sunday

Samples must be shipped so they arrive at the laboratory within 4 days of collection to be tested. Ship your samples the same day of collection to ensure your sample can be tested.

Understand Your ColoSense Result

Contact your healthcare provider to discuss your result.
The test result can be Positive, Negative, or No result obtained.

Positive Result

- **A POSITIVE result means the test detected abnormal RNA and/or blood that could be caused by cancer or precancer in the colon or rectum.**
- **A positive test result is recommended to be followed up by a colonoscopy.**
- **A false positive (incorrect) result is possible and means the test result is positive even though no cancer or precancer is present.**

Negative Result

- **A NEGATIVE result means the test did not detect abnormal RNA or blood in the sample.**
- **A negative test result does not guarantee the absence of cancer or precancer. You should continue with a regular screening schedule.**
- **A false negative (incorrect) result is possible and means the test result missed a potential cancer or precancer.**
- **Talk about your test result with your doctor.**

No Result

- **A no result or invalid result means the test was not able to produce a valid result.**
- **When this occurs, consult your doctor about next steps, which may include potentially having to provide another sample.**

ColoSense Collection Kit Symbols Used

	REF Symbol		Manufacturer Information
	Lot Number		Caution
	Expiration Date		Temperature Storage Range
	Read Instructions		Number of Tests in the Kit
	In Vitro Diagnostic Medical Device		Do Not Reuse
Rx Only	Medical Prescription		Serial Number



Geneoscopy, Inc.
2220 Welsch Industrial Ct
St. Louis, MO 63146

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ColoSense Assay Instructions for Use



For In-Vitro Diagnostic Use

Rx Only Prescription Use Only



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St. Louis, MO 63146

www.geneoscopy.com
314-887-7777

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1. INTENDED USE AND INDICATION FOR USE

ColoSense is intended for the qualitative 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 iFOBT Analyzer; bioMérieux EMAG Nucleic Acid Extraction System; and Bio-Rad QXDx 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 typical average-risk for developing CRC. ColoSense is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

For *in Vitro* diagnostic use.
For prescription use only.

2. CONTRAINDICATIONS

ColoSense is NOT indicated for use for patients who have the following:

- Personal history of colorectal cancer, adenomas, or other related cancers
- Positive result on another colorectal cancer screening method within the last six 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

3. WARNINGS AND PRECAUTIONS

- Patients should NOT provide a sample for the ColoSense test if 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, given the current data available.
 - 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 lesions.
 - A negative test result does not guarantee the absence of colorectal cancer or precancerous lesions. A false negative (incorrect) result may occur 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. Programmatic performance of ColoSense (i.e., benefits and risks with repeated testing over an established period of time) has not been studied. Non-inferiority or superiority of ColoSense programmatic 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 samples from subjects with Inflammatory Bowel Disease (IBD), 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.

4. SUMMARY AND EXPLANATION OF THE TEST

ColoSense is a multiple-target stool-RNA (mt-sRNA) based test for the presence of RNA markers and occult hemoglobin in human stool to detect colorectal cancer (CRC), advanced adenomas (AA), and serrated precancerous lesions (SPL), categorized as listed in **table 1** below. ColoSense is designed to analyze patients' stool for the presence of 8 RNA molecular biomarkers (GAPDH, ACY1, AREG, TNFRSF10B, CDH1, EGLN2, KRAS, SMAD4) and hemoglobin, supplemented with patient smoking status, which together may indicate the presence of colorectal cancer (CRC), advanced adenomas (AA), or serrated precancerous lesions (SPL). Because cellular exfoliation of RNA into stool occurs continuously, ColoSense can detect pre-malignant neoplasia at early onset.

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). A patient with a positive result should be referred for a follow-up colonoscopy. A patient with a negative result should continue with regular screening. If no result is obtained, a second stool collection may be requested.

Table 1. Classifying test results into categories based on colonoscopy findings

	Test Result	Category	Colonoscopy Findings
Colorectal Cancer (CRC)	Positive	1.0	Stage I-IV colorectal cancer, any size
Advanced Adenomas (AA)		2.1	High-grade dysplasia or ≥10 adenomas, any size
		2.2	Tubulovillous adenoma, any size
		2.3	Tubular adenoma, ≥10mm
Serrated Precancerous Lesion (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 polyps or negative lesions
		6.2	No lesions on colonoscopy

5. PRINCIPLES OF PROCEDURE

ColoSense involves stool-based testing, which detects biomarkers contained in the cells shed by individuals with colorectal cancer, advanced adenomas, or serrated precancerous lesions. The ColoSense test qualitative output is an aggregate including results of testing for RNA biomarkers, FIT/iFOBT result, and smoking status survey results. The biomarkers involve quantification of RNA expression. The FIT/iFOBT involves stool hemoglobin results.

Stool samples are 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 ColoSense Collection Kit is sent to the patient's residence after being prescribed by a healthcare provider. The patient deposits a stool sample into the ColoSense collection kit and ships the ColoSense collection kit to Geneoscopy's central-laboratory. Once the stool sample is received, the stool sample is directed into parallel workflows for analysis of hemoglobin and RNA biomarkers. To assess the hemoglobin in stool, the OC-Auto[®] sampling bottle is analyzed using an automated fecal immunochemical blood test (FIT/iFOBT) IVD system with a modified threshold. The ColoSense test evaluates 8 RNA biomarkers. Isolation and analysis of RNA 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 RNA is isolated using RNA extraction protocols, DNase treatment, and a spin column based cleanup kit per the manufacturers' instructions. Expression of the RNA biomarkers is quantified using the ColoSense Test Kit and the QXDx droplet digital PCR system. All

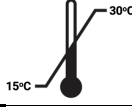
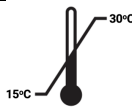
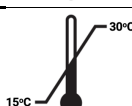
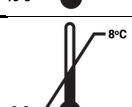
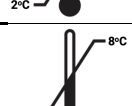
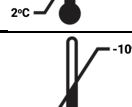
instruments and protocols are qualified for IVD use under the Geneoscopy quality system.

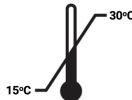
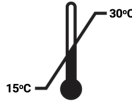
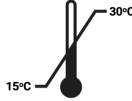
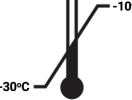
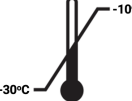
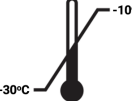
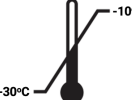
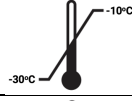
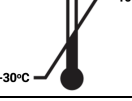
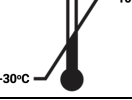
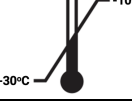
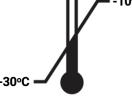
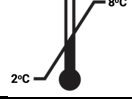
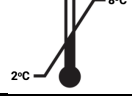
The ColoSense software is used to aggregate data from the RNA biomarkers, smoking status, and FIT/iFOBT to generate a score. The score is compared to the predefined threshold, yielding a positive (abnormal) or negative result. The score is calculated by multiplying the patient's individual RNA expression, demographic information (smoking status), and stool hemoglobin (FIT/iFOBT) results, by a marker-specific weighting factor. The aggregate of these individually weighted marker results determines the composite score, which is then compared to a cut-off to determine a positive (abnormal) or negative result. If the composite score is greater than or equal to the cut-off, the ColoSense result will be reported as positive, and the patient should be referred for a follow-up colonoscopy. If the score is below the cut-off, 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.

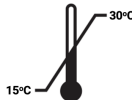
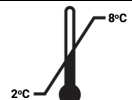
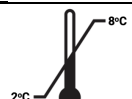
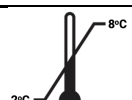
6. REAGENTS

The primary reagents required to perform the laboratory procedure for ColoSense are listed in **table 2**. For each reagent, associate process, reagent name, storage requirements, and descriptions are provided. Reagent ordering, receiving, and validation testing is performed by trained personnel according to the Geneoscopy quality system. All reagents and instruments are qualified for IVD use under the Geneoscopy quality system.

Table 2. Primary reagents required to perform ColoSense laboratory procedures

Process	Reagent Name	Storage Requirement	Description
RNA Extraction	NUCLISENS easyMAG Extraction Lysis Buffer		Guanidinium Thicyanate, Triton X-100 Surfactant
	NUCLISENS easyMAG Extraction Buffer 1		Guanidinium Thicyanate, Triton X-100 Surfactant
	NUCLISENS easyMAG Extraction Buffer 2		Morpholinoethanesulfonic acid, Proclin
	NUCLISENS easyMAG Extraction Buffer 3		Disodium Tetraborate, Anhydrous
	NUCLISENS Magnetic Silica		Paramagnetic silica, Proclin
	RQ1 RNase-free DNase		Endonuclease that digests single and double-stranded DNA

	Zymo RNA Clean and Concentrator-5		Kit containing RNA binding buffer, RNA prep buffer, RNA wash buffer, DNase I, DNA digestion buffer, DNase/RNase free water
QXDx Quantification	Automated Droplet Generation Oil for Probes		Hexane, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)-
	ddPCR Droplet Reader Oil		Hexane, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)-
	CS Assay Mix 1		Oligonucleotides with florescent bound probe
	CS Assay Mix 2		Oligonucleotides with florescent bound probe
	CS Assay Mix 3		Oligonucleotides with florescent bound probe
	CS Assay Mix 4		Oligonucleotides with florescent bound probe
	CS IPTC		gBlock DNA
	CS BTC		Water
	CS S-Mix		DNTP and DNA Polymerase solution
	CS S-Script		Reverse Transcriptase
	CS R-Out		RNase Inhibitor
FIT Quantification	OC Auto Buffer		1 x 200 mL contains 2.38 g of N-2-Hydroxyethylpiperazine-N'-2ethanesulfonic acid (HEPES)
	OC Auto Latex Reagent		Latex-sensitized anti-human HbAo rabbit IgG solution

	OC Auto Wash Concentrate		1 x 120 mL contains 5% Sodium Hypochlorite solution
	OC Auto Calibration Kit		10 x 1 mL, 2 x 45 mL Diluent
	OC Auto FOBT-CHEK Negative Control		Buffer
	OC Auto FOBT-CHEK Positive Control		Stabilized Hemoglobin suspended in a buffered, bacteriostatic and fungistatic fluid

7. PROCEDURAL WARNINGS AND PRECAUTIONS

- DO NOT use any reagent after its expiration date.
- Only use ColoSense Collection Kit for collecting samples.
- To ensure the integrity of the sample, the laboratory must receive patient specimens within 96 hours of collection.
- ColoSense reagents are intended to be used only with the ColoSense Software and instrumentation.
- Universal precautions should be used when handling human samples.
- Refer to the Safety Data Sheet (SDS) for each reagent to ensure proper disposal and safety precautions. The Instructions for Use must be followed carefully for accurate results.
- Samples must be received by the laboratory within the stability period.
- When operating laboratory equipment, always refer to the manufacturer's operating manuals for a full list of warnings and safety considerations.
- Ensure instrument settings are correct for the ColoSense Test before using any programmable instruments.
- Verify reagents are not expired prior to use.
- Ensure correct entry of information: Incorrect smoking status can impact the result.
- Use caution when labeling samples to preserve identity.
- Use caution during the test process to ensure sample identity is preserved and avoid sample mix-ups.
- Do not pipette by mouth or eat, drink or smoke in laboratory work areas.
- Avoid microbial and DNA contamination of reagents.
- Specimens should be handled as infectious using safe laboratory procedures.
- Reagents contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Invalid result could occur from improper handling or storage, technical error, or sample mix up. Ensure that only trained personnel perform the laboratory procedures.
- For additional warnings, precautions and procedures to reduce the risk of contamination, refer to the instrument manuals. Good laboratory practice and careful

adherence to the procedures specified in this document are important to reduce further risk of nucleic acid contamination from calibrators, positive controls, or specimens.

- Use standard laboratory precautions in accordance with applicable federal, state, and local regulations.
- Laboratory areas should be cleaned and maintained according to good laboratory practices for clinical laboratories processing biological specimens. Appropriate procedures shall be defined by the laboratory director, or appropriate delegate.
- Refer to user manuals for complete decontamination procedures for instruments and equipment.
- Product components (residual product, packaging, waste) can be considered laboratory waste. Dispose in accordance with applicable federal, state, and local regulations.

8. INSTRUMENTATION

The primary instruments required to perform the laboratory procedure for ColoSense are listed below. Instrumentation and supporting software are installed and qualified by trained personnel prior to training laboratory personnel. All instruments and systems are qualified and placed under the Geneoscopy quality system.

During Accessioning, centrifuges are used for pre-processing of stool samples. During RNA extraction the EMAG[®] Nucleic Acid Extraction System is used for total nucleic acid extraction and cleanup. During QXDx Quantification, the Plate Sealer and the Droplet Generator are used to set up PCR plates prior to nucleic acid amplification. The Thermal Cycler is used for PCR amplification. The Droplet Reader is used to quantify RNA biomarkers in the ColoSense test system. FIT swabs are assessed using the OC-Auto[®] Micro 80 FIT Reader. All reagents, instruments, and protocols are qualified for IVD use under the Geneoscopy quality system. **Table 3** outlines all laboratory instruments.

Table 3. Instruments required to perform ColoSense laboratory procedures

Process	Instrument	Manufacturer
Accessioning	Centrifuge	Eppendorf
	Centrifuge	Hettich
RNA Extraction	EMAG [®] Nucleic Acid Extraction System	BioMérieux
QXDx Quantification	Plate Sealer	Bio-Rad
	Thermal Cycler	Bio-Rad
	Droplet Reader	Bio-Rad
	Droplet Generator	Bio-Rad
FIT Quantification	FIT Reader	Polymedco

9. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimens for use with ColoSense must be collected with the ColoSense Collection Kit. The patient collects the stool specimen using the ColoSense Collection Kit and ships the ColoSense Collection Kit to Geneoscopy. Detailed instructions for specimen receipt and processing are outlined below. The Performance Characteristics and Interfering Substances section below provides a summary of the known substances that may impact the test results.

After sample production, stool samples are stable at ambient temperature during transit for up to 96 hours. Upon reception, accessioned stool samples can be processed to RNA immediately or can be stored at less than -60 degrees C for up to 12 months. Once an RNA extraction has been generated, specimens can be stored at less than 60 degrees C for up to 12 months.

10. COLOSENSE LABORATORY PROCEDURES

10.1. Reception and Accessioning of ColoSense Collection Kit

The patient collects a stool sample using the ColoSense Collection Kit. Stool samples are shipped directly back to Geneoscopy's laboratory as outlined in the ColoSense Collection Kit Instructions for Use. Reception and accessioning of the ColoSense Collection Kit begins the laboratory processing component of the ColoSense test system. The following steps are required for ColoSense Reception and Accessioning:

- Receive the ColoSense Collection Kit using external kit identifiers.
- Confirm the collection date is within the stability period.
- Remove ColoSense Collection Kit contents:
 - Retain all sample materials (stool sample and FIT swab),
 - Retain records (attestation form) and record required information,
 - Discard any remaining components in accordance with local regulations.
- Evaluate the stool sample to determine if sample quantity and quality is sufficient.
- Homogenize the sample, if needed.
- Aliquot up to 40mL of stool homogenate into conical tubes and spin down using a centrifuge.
- When complete, remove samples from the centrifuge and decant the supernatant to generate retained eukaryotic cell fractions.
- Eukaryotic cell fractions can be processed immediately or stored per stability claims.

10.2. RNA Extraction of Stool Samples using the ColoSense Test System

At the end of Reception and Accessioning, eukaryotic cell fractions are either processed immediately, or frozen for storage and delayed processing. These eukaryotic cell fractions undergo RNA Extraction to isolate clean solutions of total RNA that are ready for RNA Quantification. The following steps are required for RNA Extraction:

- For each sample being processed, obtain eukaryotic cell fraction tubes.
- Add EasyMag Lysis Buffer to each eukaryotic cell fraction tube.
- Resuspend each stool eukaryotic fraction in the Lysis Buffer.
- Centrifuge the tubes.
- Remove the tubes from the centrifuge after the spin is complete.
- Combine the supernatant from both lysed eukaryotic cell fractions into a new conical tube.
 - Lysates can be processed immediately or the lysate can be stored prior to starting the RNA extraction protocol.
- To process lysates, pipette lysate into EMAG cartridges and perform the RNA extraction protocol.
- Following the sample elution, remove the samples from the EMAG and proceed to DNase treatment.
- Prepare DNase solution according to manufacturing instructions.
- Add DNase to each sample, and seal sample tubes.
- Incubate samples on the heat device.
- At completion of incubation, remove samples from the heat device.
- Following DNase treatment, pipette samples into EMAG cartridges and perform the RNA cleanup protocol.
- Following the sample elution, remove the samples from the machine and proceed to transfer tube treatment.
- Perform transfer tube protocol according to the manufacturer instructions.
- After transfer tube protocol, the final solution (total RNA extractions) can be processed immediately or stored per stability claims.

10.3. RNA Quantification using the ColoSense Test System

At the end of RNA Quantification, total RNA can be either processed immediately, or frozen for storage and delayed processing. Total RNA undergoes RNA quantification to generate concentration values for the RNA biomarkers in the ColoSense assay and serve as inputs into the ColoSense Algorithm. The following steps are required for RNA Quantification:

- Place total RNA inputs on ice.
- Retrieve a ColoSense Test Kit from storage.
- Thaw the ColoSense Test Kit at room temperature.
- Generate mastermix components for each of the 4 Assay Mixes for the 96-well plate.
- Vortex all components.
- Set 96-well plate into an IsoFreeze PCR rack and pipette mastermix into the 96-well plate.
- Add the RNA samples or controls into appropriate wells.
- Seal the plate using a plate seal.
- Load consumables onto the Automated Droplet Generator.
- Place input 96-well PCR plate in the appropriate slot on the droplet generator.
- Press the “Start Droplet Generation Button” to launch the Automated Droplet Generator.
- Following completion of the run, remove the output 96-well plate and proceed immediately to plate sealing.
- Ensure that the PX1 Plate Sealer displays proper temperature and time.

- Place a foil seal on top of the plate and seal the plate.
- Following plate sealing, proceed immediately to Thermal Cycling.
- Place the sealed 96-well plate in the Thermal Cycler
- Select the ColoSense Protocol from the list of saved files and start the run.
- Following completion of the thermal cycling protocol, the plate can be read immediately on the Droplet Reader or stored prior to droplet reading.
- Launch the QuantaSoft software and select the ColoSense Assay template.
- Add sample IDs to the ColoSense Assay template.
- Load the 96-well plate onto the Droplet Reader.
- Following the run, save the plate data for automatic upload.

10.4. FIT Quantification using the ColoSense Test System

FIT swabs are obtained from the ColoSense Collection Kit during Reception and Accessioning. FIT swabs are either processed immediately or stored at reduced temperatures for delayed processing. FIT swabs undergo quantification to generate hemoglobin concentration values, which serves as an input into the ColoSense Algorithm. The following steps are required for FIT Quantification:

- Check all reagents to ensure they are not expired.
 - Replace buffer, wash solution, and deionized water if needed.
 - The buffer must be incubated at room temperature prior to use.
 - Wash solution concentrate must be diluted with deionized water prior to use.
 - Latex reagent must be uncapped and installed on the left-hand side of the reagent cradle prior to each use.
 - Following reagent replacement, the instrument must be primed.
- Ensure appropriate calibrations are completed prior to use.
 - To calibrate the instrument, reconstitute the calibrator reagent using deionized water, generate the calibration serial dilutions using the dilution cups, and run the calibration curve protocol.
- Ensure appropriate control testing is completed prior to use.
 - To complete control testing, run a positive control and a negative control in a single run and compare results to control labels.
- Once controls and calibrators are completed, sample FITs can be assessed.
- Load sampling bottles into a sample rack, add empty cuvettes, and run the instrument.
- Results will be generated and stored automatically.
- Discard all reagents in accordance with local regulations.

11. DATA HANDLING AND ANALYSIS

All data required to generate a ColoSense result is processed using the ColoSense software. Data from the molecular component and the FIT component are entered into the LIMS to maintain traceability of the sample to result throughout the testing process. Users review, comment upon, or invalidate sample or run data in the software as required to capture any errors or invalid samples that occur during the assay procedure.

The data from the molecular component, the FIT component, and the patients smoking status are entered in the ColoSense Software which calculates the overall ColoSense score for each sample.

Once the test runs are complete, the run data is imported into the ColoSense Software. This information includes: 1) the FIT component 2) patient smoking status and 3) normalized stool-derived eukaryotic RNA transcript concentrations. In total, these input features are processed using the ColoSense Software to generate a ColoSense result. A Negative or Positive result is generated based on the ColoSense score. If any of the individual input results are invalid, it will lead to an Invalid ColoSense result, resulting in a No Result.

After the ColoSense Software generates the result, a patient report is created which contains all pertinent information to identify the patient and convey the ColoSense result. The patient report is sent to the healthcare provider so that the result can be communicated to the patient.

12. INTERPRETATION OF RESULTS

Once a composite score is generated, the ColoSense software imports the result into the Laboratory Information Management System (LIMS). For each sample, data in the LIMS is reviewed prior to the release of the ColoSense result. This can include evaluating controls, verifying pertinent information, and disposition of process deviations or non-conformances. If a deviation (e.g., operator error, instrument error) occurs, the deviation may compromise the results of the test regardless of the control validity. In the event that a specimen result is compromised (e.g., contamination, processing error), the individual result may be invalidated.

Valid ColoSense results can be one of the following: Positive, Negative, or No Result. After LIMS data is reviewed, results can be released and reported. A positive ColoSense result may indicate the presence of colorectal cancer, advanced adenomas, and/or serrated precancerous lesions and should be followed by a colonoscopy. A negative result indicates that the patient can continue with regular screening. A No Result ColoSense result occurs if the specimen result from any constituent assay is invalid. If no result is obtained, a subsequent stool collection may be requested.

13. QUALITY CONTROL

Required controls must be assessed for both the molecular component and the FIT component prior to disposition of results.

- For the molecular component, a positive control (IPTC) and a negative control (BTC) are provided with every ColoSense Test Kit. The IPTC and BTC are run on each 96-well plate with samples.
- For the FIT component, a positive control (solution with hemoglobin) and a negative control (deionized water) are provided by the manufacturer. The positive and negative controls must be run within the last 24-hours prior to assessing samples.

Process controls must yield expected results, or the assay run will be invalid.

Users are responsible for tracking sample IDs and documentation of processing errors. Users should review and comment upon overall ColoSense results and invalidate sample results, as needed, based on events and issues known to the user. Sample results invalidated during previous review steps will be called 'Invalid.' If a sample is invalidated, stored samples can be used for reprocessing, or a subsequent stool collection may be requested.

14. LIMITATIONS OF THE ASSAY

- The ColoSense Test is intended only for testing of colorectal neoplasia in fecal samples.
- ColoSense is NOT indicated for patients who have the following:
 - Personal history of colorectal cancer, adenomas, or other related cancers
 - Positive result on another colorectal cancer screening method within the last six 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
- As with any screening test, results obtained with the ColoSense Test should not be considered conclusive evidence of the presence or absence of colorectal neoplasia. ColoSense is designed for screening and not intended to replace other diagnostic procedures such as colonoscopy.
- The presence of foreign materials in the sample (e.g., urine or toilet paper) may interfere with test results.
- Incorrect designation of the smoking status may lead to incorrect results.
- Colosense is not intended for use in testing urine, blood, or other body fluids.
- A negative test result does not exclude the possibility that the patient may have a precancerous or cancerous polyp. A false negative result with ColoSense could potentially delay colonoscopy and a potentially delayed diagnosis of disease.
- A positive ColoSense result suggests the presence of precancerous polyps and/or cancer. A false positive result could result in an additional invasive screening procedure for the patient, such as colonoscopy, and thus expose patients to the risks associated with such a procedure.

15. PERFORMANCE CHARACTERISTICS

15.1. Clinical Cutoff

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 colonoscopy¹. 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 disease². In total, 1,327 participants were evaluated for 8 stool-derived eukaryotic RNA biomarkers, FIT result, and demographic information (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). When used on the hold out testing set, the mt-sRNA test attained a 95% sensitivity for colorectal cancer (n = 22), 62% sensitivity for advanced adenomas and other advanced precancerous lesions (n = 52), 25% sensitivity for non-advanced adenomas (n = 139), 80% specificity for hyperplastic polyps (n = 74), and 85% specificity for no findings on a colonoscopy (n = 101). Final model development was performed by quantifying the entire cohort with the final panel and retraining the ordinal regression model. In total, there were 1,114 samples that were employed in final model development. The optimal threshold corresponded to the 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.

15.2. ColoSense Analytical Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantification (LoQ) studies were performed for the molecular component of the ColoSense test based on guidance from the CLSI Standard: EP17-A (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 was 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 $\leq 20\%$ 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.

A Contrived Specimen Functional Equivalency Study (CSFS) was performed to demonstrate equivalency between clinical specimens and contrived specimens that were leveraged as part of the LoD/LoQ studies. Clinical specimens were obtained from subjects and 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 the classical analysis. LoDs derived using clinical specimens were compared to those derived using contrived specimens to ensure functional equivalency.

Analytical sensitivity characteristics for ColoSense are provided in **table 4** below:

Table 4. Performance characteristics for LoD and LoQ

Performance Characteristic	Result
Limit of Blank	All Markers: 0.00 - 0.53 copies/μL
Limit of Detection	All Markers: 0.31 - 0.66 copies/μL
Limit of Quantification	All Markers: 1.29 - 2.55 copies/μL

15.3. ColoSense Test Specificity and Cross-Reactivity

The potential for off-target binding of the molecular component of the ColoSense test was evaluated using an in-silico analysis. Off-target binding was evaluated for human, enterobacterial, and viral transcripts.

The study results demonstrated the molecular component of the ColoSense test is specific to the targets of interest with no potential for off-target amplification from non-specific targets.

The potential for cross-reactivity with non-colorectal cancers and inflammatory conditions was evaluated by testing 131 subjects across 14 different diseases (**table 5**). 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. 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 Inflammatory Bowel Disease, breast cancer, gynecological cancers, prostate cancer, and lipoma, and average of six positive calls per 10,000 screening patients tested across all tested diseases.

Table 5. ColoSense positivity rates for non-CRC diseases and cancers

Disease	Samples tested	Incidence rate per 10,000 ^a	% ColoSense positive	Projected positive calls per 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
Gynecologic Cancer ^{b,d}	6	4.89	50.0	1.2 ^d

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
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.

15.4. Precision and Reproducibility

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, stool replicates for four 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 stool samples per score group were evaluated. For each stool sample, a total of 3 unique replicates were assessed. Each of the 3 replicates were assessed on 3 unique days, by 3 unique operators, 3 unique QXDx instrument combinations and 3 unique lots of reagent.

Determination of precision based on each sample score group is noted in **table 6**. 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 6. Overall ColoSense precision for clinical replicates

Score Group	Replicates	Mean	Standard Deviation	%CV	% (n/N)
Low Negative	8	0.076	0.0029	3.9%	100% (24/24)
High Negative	8	0.100	0.0046	4.6%	100% (24/24)
Low Positive	8	0.123	0.0046	3.7%	100% (24/24)
High Positive	8	0.220	0.0118	5.4%	100% (24/24)

ColoSense precision for operator-to-operator (**table 7**), lot-to-lot (**table 8**), equipment-to-equipment (**table 9**), day-to-day (**table 10**), run-to-run (**table 11**), and within runs (**table 12**), 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 a total of 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).

Table 7. ColoSense precision (operator-to-operator)

Score Group	Operator	Result	Acceptance Criteria	% (n/N)
Low Negative	Operator 1	100% concordance	Concordance > 95%	100% (36/36)
	Operator 2	100% concordance	Concordance > 95%	100% (36/36)
	Operator 3	100% concordance	Concordance > 95%	100% (36/36)
High Negative	Operator 1	1.90% CV	% CV ≤ 20%	100% (35/35)
	Operator 2	2.31% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	2.10% CV	% CV ≤ 20%	100% (36/36)
Low Positive	Operator 1	5.33% CV	% CV ≤ 20%	100% (36/36)
	Operator 2	4.41% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	3.93% CV	% CV ≤ 20%	100% (35/35)
High Positive	Operator 1	11.04% CV	% CV ≤ 20%	100% (36/36)
	Operator 2	6.24% CV	% CV ≤ 20%	100% (36/36)
	Operator 3	6.08% CV	% CV ≤ 20%	100% (36/36)

Table 8. ColoSense precision (lot-to-lot)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
Low Negative	Lot 1	100% concordance	Concordance > 95%	100% (36/36)
	Lot 2	100% concordance	Concordance > 95%	100% (36/36)
	Lot 3	100% concordance	Concordance > 95%	100% (36/36)
High Negative	Lot 1	2.17% CV	% CV ≤ 20%	100% (36/36)
	Lot 2	1.99% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	1.73% CV	% CV ≤ 20%	100% (35/35)
Low Positive	Lot 1	5.59% CV	% CV ≤ 20%	100% (35/35)
	Lot 2	3.17% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	4.86% CV	% CV ≤ 20%	100% (36/36)
High Positive	Lot 1	12.06% CV	% CV ≤ 20%	100% (36/36)
	Lot 2	4.73% CV	% CV ≤ 20%	100% (36/36)
	Lot 3	6.84% CV	% CV ≤ 20%	100% (36/36)

Table 9. ColoSense precision (equipment-to-equipment)

Target Level	Equipment	Result	Acceptance Criteria	% (n/N)
Low Negative	System 1	100% concordance	Concordance > 95%	100% (24/24)
	System 2	100% concordance	Concordance > 95%	100% (36/36)
	System 3	100% concordance	Concordance > 95%	100% (24/24)
	System 4	100% concordance	Concordance > 95%	100% (24/24)
High Negative	System 1	1.86% CV	% CV ≤ 20%	100% (23/23)
	System 2	2.10% CV	% CV ≤ 20%	100% (36/36)
	System 3	2.60% CV	% CV ≤ 20%	100% (24/24)
	System 4	1.92% CV	% CV ≤ 20%	100% (24/24)
Low Positive	System 1	5.83% CV	% CV ≤ 20%	100% (23/23)
	System 2	4.72% CV	% CV ≤ 20%	100% (36/36)
	System 3	3.02% CV	% CV ≤ 20%	100% (24/24)
	System 4	4.08% CV	% CV ≤ 20%	100% (24/24)
	System 1	12.70% CV	% CV ≤ 20%	100% (24/24)

Target Level	Equipment	Result	Acceptance Criteria	% (n/N)
High Positive	System 2	6.32% CV	% CV ≤ 20%	100% (36/36)
	System 3	5.04% CV	% CV ≤ 20%	100% (24/24)
	System 4	7.68% CV	% CV ≤ 20%	100% (24/24)

Table 10. ColoSense precision (day-to-day)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
Low Negative	Day 1	100% concordance	Concordance > 95%	100% (36/36)
	Day 2	100% concordance	Concordance > 95%	100% (36/36)
	Day 3	100% concordance	Concordance > 95%	100% (36/36)
High Negative	Day 1	2.17% CV	% CV ≤ 20%	100% (36/36)
	Day 2	1.99% CV	% CV ≤ 20%	100% (36/36)
	Day 3	1.73% CV	% CV ≤ 20%	100% (35/35)
Low Positive	Day 1	5.89% CV	% CV ≤ 20%	100% (35/35)
	Day 2	3.17% CV	% CV ≤ 20%	100% (36/36)
	Day 3	4.86% CV	% CV ≤ 20%	100% (36/36)
High Positive	Day 1	12.06% CV	% CV ≤ 20%	100% (36/36)
	Day 2	4.73% CV	% CV ≤ 20%	100% (36/36)
	Day 3	6.84% CV	% CV ≤ 20%	100% (36/36)

Table 11. ColoSense precision (run-to-run)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
Low Negative	Run 1	100% concordance	Concordance > 95%	100% (12/12)
	Run 2	100% concordance	Concordance > 95%	100% (12/12)
	Run 3	100% concordance	Concordance > 95%	100% (12/12)
	Run 4	100% concordance	Concordance > 95%	100% (12/12)
	Run 5	100% concordance	Concordance > 95%	100% (12/12)
	Run 6	100% concordance	Concordance > 95%	100% (12/12)
	Run 7	100% concordance	Concordance > 95%	100% (11/11)
	Run 8	100% concordance	Concordance > 95%	100% (12/12)
	Run 9	100% concordance	Concordance > 95%	100% (12/12)
High Negative	Run 1	2.16% CV	% CV ≤ 20%	100% (12/12)
	Run 2	2.43% CV	% CV ≤ 20%	100% (12/12)
	Run 3	1.82% CV	% CV ≤ 20%	100% (12/12)
	Run 4	2.17% CV	% CV ≤ 20%	100% (12/12)
	Run 5	1.90% CV	% CV ≤ 20%	100% (12/12)
	Run 6	2.03% CV	% CV ≤ 20%	100% (12/12)
	Run 7	1.48% CV	% CV ≤ 20%	100% (12/12)
	Run 8	2.14% CV	% CV ≤ 20%	100% (12/12)
	Run 9	0.90% CV	% CV ≤ 20%	100% (12/12)
Low Positive	Run 1	7.69% CV	% CV ≤ 20%	100% (11/11)
	Run 2	5.12% CV	% CV ≤ 20%	100% (12/12)
	Run 3	2.61% CV	% CV ≤ 20%	100% (12/12)
	Run 4	3.36% CV	% CV ≤ 20%	100% (12/12)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
	Run 5	3.62% CV	% CV ≤ 20%	100% (12/12)
	Run 6	2.57% CV	% CV ≤ 20%	100% (12/12)
	Run 7	4.68% CV	% CV ≤ 20%	100% (12/12)
	Run 8	4.11% CV	% CV ≤ 20%	100% (12/12)
	Run 9	5.41% CV	% CV ≤ 20%	100% (12/12)
High Positive	Run 1	16.18% CV	% CV ≤ 20%	100% (12/12)
	Run 2	8.24% CV	% CV ≤ 20%	100% (12/12)
	Run 3	5.38% CV	% CV ≤ 20%	100% (12/12)
	Run 4	3.19% CV	% CV ≤ 20%	100% (12/12)
	Run 5	5.74% CV	% CV ≤ 20%	100% (12/12)
	Run 6	4.78% CV	% CV ≤ 20%	100% (12/12)
	Run 7	5.32% CV	% CV ≤ 20%	100% (12/12)
	Run 8	5.36% CV	% CV ≤ 20%	100% (12/12)
	Run 9	9.33% CV	% CV ≤ 20%	100% (12/12)

Table 12. ColoSense precision (within run)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
Low Negative	Run 1	100% concordance	Concordance > 95%	100% (4/4)
	Run 2	100% concordance	Concordance > 95%	100% (4/4)
	Run 3	100% concordance	Concordance > 95%	100% (4/4)
	Run 4	100% concordance	Concordance > 95%	100% (4/4)
	Run 5	100% concordance	Concordance > 95%	100% (4/4)
	Run 6	100% concordance	Concordance > 95%	100% (4/4)
	Run 7	100% concordance	Concordance > 95%	100% (4/4)
	Run 8	100% concordance	Concordance > 95%	100% (4/4)
	Run 9	100% concordance	Concordance > 95%	100% (4/4)
	Run 10	100% concordance	Concordance > 95%	100% (4/4)
	Run 11	100% concordance	Concordance > 95%	100% (4/4)
	Run 12	100% concordance	Concordance > 95%	100% (4/4)
	Run 13	100% concordance	Concordance > 95%	100% (4/4)
	Run 14	100% concordance	Concordance > 95%	100% (4/4)
	Run 15	100% concordance	Concordance > 95%	100% (4/4)
	Run 16	100% concordance	Concordance > 95%	100% (4/4)
	Run 17	100% concordance	Concordance > 95%	100% (4/4)
	Run 18	100% concordance	Concordance > 95%	100% (4/4)
	Run 19	100% concordance	Concordance > 95%	100% (4/4)
	Run 20	100% concordance	Concordance > 95%	100% (4/4)
	Run 21	100% concordance	Concordance > 95%	100% (4/4)
	Run 22	100% concordance	Concordance > 95%	100% (4/4)
	Run 23	100% concordance	Concordance > 95%	100% (4/4)
	Run 24	100% concordance	Concordance > 95%	100% (4/4)
	Run 25	100% concordance	Concordance > 95%	100% (4/4)
	Run 26	100% concordance	Concordance > 95%	100% (4/4)
	Run 27	100% concordance	Concordance > 95%	100% (4/4)
	Run 1	1.76% CV	% CV ≤ 20%	100% (4/4)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
High Negative	Run 2	1.73% CV	% CV ≤ 20%	100% (4/4)
	Run 3	2.42% CV	% CV ≤ 20%	100% (4/4)
	Run 4	0.79% CV	% CV ≤ 20%	100% (4/4)
	Run 5	0.67% CV	% CV ≤ 20%	100% (4/4)
	Run 6	2.15% CV	% CV ≤ 20%	100% (4/4)
	Run 7	1.18% CV	% CV ≤ 20%	100% (3/3)
	Run 8	2.02% CV	% CV ≤ 20%	100% (4/4)
	Run 9	1.11% CV	% CV ≤ 20%	100% (4/4)
	Run 10	3.53% CV	% CV ≤ 20%	100% (4/4)
	Run 11	2.85% CV	% CV ≤ 20%	100% (4/4)
	Run 12	1.72% CV	% CV ≤ 20%	100% (4/4)
	Run 13	1.95% CV	% CV ≤ 20%	100% (4/4)
	Run 14	0.70% CV	% CV ≤ 20%	100% (4/4)
	Run 15	1.10% CV	% CV ≤ 20%	100% (4/4)
	Run 16	0.80% CV	% CV ≤ 20%	100% (4/4)
	Run 17	2.70% CV	% CV ≤ 20%	100% (4/4)
	Run 18	0.70% CV	% CV ≤ 20%	100% (4/4)
	Run 19	1.08% CV	% CV ≤ 20%	100% (4/4)
	Run 20	2.50% CV	% CV ≤ 20%	100% (4/4)
	Run 21	1.28% CV	% CV ≤ 20%	100% (4/4)
	Run 22	1.65% CV	% CV ≤ 20%	100% (4/4)
	Run 23	3.11% CV	% CV ≤ 20%	100% (4/4)
	Run 24	1.60% CV	% CV ≤ 20%	100% (4/4)
	Run 25	1.85% CV	% CV ≤ 20%	100% (4/4)
	Run 26	2.18% CV	% CV ≤ 20%	100% (4/4)
	Run 27	0.73% CV	% CV ≤ 20%	100% (4/4)
Low Positive	Run 1	8.64% CV	% CV ≤ 20%	100% (4/4)
	Run 2	4.06% CV	% CV ≤ 20%	100% (4/4)
	Run 3	1.20% CV	% CV ≤ 20%	100% (4/4)
	Run 4	3.09% CV	% CV ≤ 20%	100% (4/4)
	Run 5	4.15% CV	% CV ≤ 20%	100% (4/4)
	Run 6	1.91% CV	% CV ≤ 20%	100% (4/4)
	Run 7	3.82% CV	% CV ≤ 20%	100% (4/4)
	Run 8	4.06% CV	% CV ≤ 20%	100% (4/4)
	Run 9	3.70% CV	% CV ≤ 20%	100% (4/4)
	Run 10	3.65% CV	% CV ≤ 20%	100% (4/4)
	Run 11	3.00% CV	% CV ≤ 20%	100% (4/4)
	Run 12	2.79% CV	% CV ≤ 20%	100% (4/4)
	Run 13	2.11% CV	% CV ≤ 20%	100% (4/4)
	Run 14	1.19% CV	% CV ≤ 20%	100% (4/4)
	Run 15	2.85% CV	% CV ≤ 20%	100% (4/4)
	Run 16	5.39% CV	% CV ≤ 20%	100% (4/4)
	Run 17	4.77% CV	% CV ≤ 20%	100% (4/4)
	Run 18	8.70% CV	% CV ≤ 20%	100% (4/4)
	Run 19	1.45% CV	% CV ≤ 20%	100% (3/3)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
	Run 20	7.33% CV	% CV ≤ 20%	100% (4/4)
	Run 21	2.80% CV	% CV ≤ 20%	100% (4/4)
	Run 22	5.00% CV	% CV ≤ 20%	100% (4/4)
	Run 23	4.45% CV	% CV ≤ 20%	100% (4/4)
	Run 24	2.68% CV	% CV ≤ 20%	100% (4/4)
	Run 25	3.32% CV	% CV ≤ 20%	100% (4/4)
	Run 26	3.51% CV	% CV ≤ 20%	100% (4/4)
	Run 27	1.74% CV	% CV ≤ 20%	100% (4/4)
High Positive	Run 1	10.87% CV	% CV ≤ 20%	100% (4/4)
	Run 2	4.87% CV	% CV ≤ 20%	100% (4/4)
	Run 3	4.48% CV	% CV ≤ 20%	100% (4/4)
	Run 4	4.21% CV	% CV ≤ 20%	100% (4/4)
	Run 5	9.15% CV	% CV ≤ 20%	100% (4/4)
	Run 6	4.66% CV	% CV ≤ 20%	100% (4/4)
	Run 7	5.56% CV	% CV ≤ 20%	100% (4/4)
	Run 8	3.35% CV	% CV ≤ 20%	100% (4/4)
	Run 9	5.62% CV	% CV ≤ 20%	100% (4/4)
	Run 10	7.27% CV	% CV ≤ 20%	100% (4/4)
	Run 11	5.95% CV	% CV ≤ 20%	100% (4/4)
	Run 12	4.94% CV	% CV ≤ 20%	100% (4/4)
	Run 13	2.09% CV	% CV ≤ 20%	100% (4/4)
	Run 14	5.07% CV	% CV ≤ 20%	100% (4/4)
	Run 15	2.42% CV	% CV ≤ 20%	100% (4/4)
	Run 16	4.66% CV	% CV ≤ 20%	100% (4/4)
	Run 17	6.86% CV	% CV ≤ 20%	100% (4/4)
	Run 18	12.87% CV	% CV ≤ 20%	100% (4/4)
	Run 19	9.55% CV	% CV ≤ 20%	100% (4/4)
	Run 20	11.35% CV	% CV ≤ 20%	100% (4/4)
	Run 21	6.13% CV	% CV ≤ 20%	100% (4/4)
	Run 22	4.13% CV	% CV ≤ 20%	100% (4/4)
	Run 23	2.67% CV	% CV ≤ 20%	100% (4/4)
	Run 24	4.60% CV	% CV ≤ 20%	100% (4/4)
	Run 25	5.71% CV	% CV ≤ 20%	100% (4/4)
	Run 26	4.59% CV	% CV ≤ 20%	100% (4/4)
	Run 27	9.72% CV	% CV ≤ 20%	100% (4/4)

15.5. Robustness

The molecular portion of the ColoSense test involves 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.

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 each step (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 (4 positive scores and 4 negative scores). 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.

15.6. 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.

15.7. Carry-over and Cross-contamination ColoSense Testing

Potential carry-over contamination for the molecular component of the ColoSense test was evaluated using sequential runs of samples with high positive and low negative scores. Testing involved 1 run of low negative samples, followed by 2 consecutive runs of high positive samples and 1 final run of low negative samples. Each run consisted of 22 samples at the appropriate ColoSense score level. All samples were control samples consisting of synthetic RNA. Results from the molecular carry-over contamination analysis demonstrated no carry-over present in the ColoSense test.

Cross-contamination testing of the molecular component of the ColoSense test was performed using a checkerboard plate layout, alternating samples with high

positive and low negative ColoSense scores to evaluate the potential for cross-contamination during digital PCR. All samples were control samples consisting of synthetic RNA. The checkerboard plate layout contained 11 high positive and 11 low negative samples. ColoSense results from the checkerboard plate were compared to a plate containing only low negative samples. The results from the cross-contamination study for the molecular component of the ColoSense test determined that the test and associated instrumentation are not impacted by cross-contamination.

15.8. FIT/iFOBT Precision

FIT/iFOBT precision studies were performed using the OC-Auto® Sampling Bottle (Polymedco) and OC-Auto Micro 80 Instrument (Polymedco) to support the ColoSense cutoff. A precision and 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 two (2) operators, using three (3) lots and two (2) systems.

Overall, the FIT/iFOBT component of the ColoSense test had percent concordance greater than 95% or %CV less than 20% for all score levels (**table 13**). The FIT/iFOBT component of the ColoSense test results assessing operator-to-operator (**table 14**), lot-to-lot (**table 15**), equipment-to-equipment (**table 16**) are also shown below.

Table 13. Overall FIT precision for clinical replicates

Target Level	Result	Acceptance Criteria	% (n/N)
Low Negative	100% concordance	concordance > 95%	100% (240/240)
High Negative	12.6 % CV	% CV ≤ 20%	94% (225/240)
Low Positive	9.3 % CV	% CV ≤ 20%	90% (216/240)
High Positive	10.6 % CV	% CV ≤ 20%	99% (238/240)

Table 14. FIT precision (operator-to-operator)

Target Level	Operator	Result	Acceptance Criteria	% (n/N)
Low Negative	Operator 1	100% concordance	concordance > 95%	100% (120/120)
	Operator 2	100% concordance	concordance > 95%	100% (120/120)
High Negative	Operator 1	12.0% CV	% CV ≤ 20%	100% (120/120)
	Operator 2	11.0% CV	% CV ≤ 20%	88% (105/120)
Low Positive	Operator 1	9.1% CV	% CV ≤ 20%	86% (103/120)
	Operator 2	8.9% CV	% CV ≤ 20%	94% (109/116)
High Positive	Operator 1	11.1% CV	% CV ≤ 20%	98% (118/120)
	Operator 2	8.8% CV	% CV ≤ 20%	100% (120/120)

Table 15. FIT precision (lot-to-lot)

Target Level	Lot	Result	Acceptance Criteria	% (n/N)
Low Negative	Lot 1	100% concordance	concordance > 95%	100% (80/80)
	Lot 2	100% concordance	concordance > 95%	100% (80/80)
	Lot 3	100% concordance	concordance > 95%	100% (80/80)
High Negative	Lot 1	12.3% CV	% CV ≤ 20%	91% (73/80)
	Lot 2	12.3% CV	% CV ≤ 20%	99% (79/80)
	Lot 3	12.9% CV	% CV ≤ 20%	92% (73/80)
Low Positive	Lot 1	10.7% CV	% CV ≤ 20%	88% (70/80)
	Lot 2	9.3% CV	% CV ≤ 20%	84% (67/80)
	Lot 3	7.2% CV	% CV ≤ 20%	98% (75/76)
High Positive	Lot 1	8.8% CV	% CV ≤ 20%	100% (80/80)
	Lot 2	8.0% CV	% CV ≤ 20%	98% (78/80)
	Lot 3	11.5% CV	% CV ≤ 20%	100% (80/80)

Table 16. FIT precision (equipment-to-equipment)

Target Level	System	Result	Acceptance Criteria	% (n/N)
Low Negative	System 1	100% concordance	concordance > 95%	100% (120/120)
	System 2	100% concordance	concordance > 95%	100% (120/120)
High Negative	System 1	13.5% CV	% CV ≤ 20%	93% (111/120)
	System 2	11.5% CV	% CV ≤ 20%	95% (114/120)
Low Positive	System 1	9.0% CV	% CV ≤ 20%	88% (105/120)
	System 2	9.5% CV	% CV ≤ 20%	92% (107/116)
High Positive	System 1	11.2% CV	% CV ≤ 20%	98% (118/120)
	System 2	9.1% CV	% CV ≤ 20%	100% (120/120)

15.9. Stability Studies

Sample Preservative Buffer Stability

Real time stability testing of the ColoSense Sample Preservative was conducted by evaluating the functional performance of three (3) sample preservative lots over a period of 15 months. Each lot consisted of unique batches of reagents which were tested at various time points over the 15-month period. Samples used to evaluate the ColoSense Sample Preservative component were stool

specimens collected within 96 hours of RNA extraction. Fourteen RNA replicates were evaluated per lot of ColoSense Sample Preservative at each time point. In the real time stability study for the ColoSense Sample Preservative, all time points met acceptance criteria. The data supported the twelve (12) month shelf life currently assigned to the ColoSense Sample Preservative.

Test Kit Storage Stability

Real time stability testing of the ColoSense test kit for the molecular component of the test was conducted by evaluating the functional performance of three (3) test kit lots over a period of 13 months. Each lot consisted of unique batches of reagents which were tested at various time points over the 13-month period. 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 QC testing of the ColoSense test kit. At each time point, replicates of each control or mixture were tested. The results of the ColoSense test kit real time stability study met predetermined acceptance criteria. The data supported the ten (10) month shelf life currently assigned to the ColoSense test kit.

Test Kit Freeze-Thaw Stability

Freeze-thaw stability testing was conducted on the ColoSense test kit for the molecular component of the test. Freeze-thaw stability was determined by evaluating the functional performance of three (3) test kit lots over a period of three (3) freeze-thaw cycles. Each lot consisted of unique batches of reagents which were tested at three freeze-thaw cycles. Samples used to evaluate the molecular test component were a synthetic DNA control provided as part of the ColoSense test kit and synthetic RNA mixtures required for QC testing of the ColoSense Test Kit. After each freeze-thaw cycle, replicates of each control or mixture were tested. The results of the ColoSense test kit freeze-thaw stability study met predetermined acceptance criteria. The data demonstrated that the ColoSense test kit is stable for two (2) freeze-thaw events.

Collection Kit Testing

The following additional studies were conducted for the ColoSense collection kit:

- Shipping stability and drop testing to demonstrate the collection kit can withstand typical forces encountered during shipment to and from the user and laboratory.
- Sample stability testing to demonstrate the collection kit can preserve samples for the claimed time periods.
- Biocompatibility testing to demonstrate the kit components are compatible with human use.
- Human factors studies demonstrating patients can successfully use the collection kit in an at-home environment.

All studies performed on the ColoSense collection kit met acceptance criteria.

FIT/iFOBT Stability

FIT/iFOBT stability studies were performed using the OC-Auto® Sampling Bottle (Polymedco) and OC-Auto Micro 80 Instrument (Polymedco) to support the ColoSense cutoff. FIT stability studies were conducted to demonstrate that the OC-Auto Sample Bottle is stable for 96-hours at ambient temperature plus 30 days of storage between 2-8°C. All studies performed on FIT stability testing met acceptance criteria.

15.10. Clinical Sensitivity and Specificity

The pivotal study, ColoRectal Cancer and PRE-Cancerous Adenoma Non-Invasive Detection Test (CRC-PREVENT), was leveraged to assess safety and efficacy of the ColoSense test. The primary objective of the CRC-PREVENT study was to determine sensitivity and specificity of ColoSense using colonoscopy as the reference method.³

Study Design

The phase 3 clinical trial (CRC-PREVENT) was a blinded, prospective, cross-sectional study to support a premarket approval application for a class III medical device. Study subjects were identified online using social media platforms and enrolled from June 2021 to June 2022 using a decentralized recruitment model. All participants completed the ColoSense test, which incorporated a commercially available fecal immunochemical test (FIT), concentration of 8 RNA transcripts, and participant-reported smoking status. Stool samples were collected prior to participants completing a colonoscopy at their local endoscopy center. The ColoSense test results (positive or negative) were compared with index lesions observed on colonoscopy. Index lesions were confirmed by histopathologic examination using **table 1**.

Inclusion and Exclusion Criteria

Interested participants completed a self-reported online survey to screen for the intended use population and to obtain socioeconomic and demographic information. Subjects were considered eligible for the study if they were male or female, aged 45 or older, and qualified for Average-risk, asymptomatic colorectal cancer screening. Per the clinical trial protocol, a subset of Non-Average-risk participants (e.g., family history of colorectal cancer or negative noninvasive screening test result) was permitted for inclusion, despite falling outside the intended use population. Participants were excluded if they reported a personal history of colorectal neoplasia, digestive cancer, a history of high-risk conditions (e.g., hereditary cancer syndromes and inflammatory bowel disease), overt rectal bleeding within the last 30 days, undergoing a colonoscopy within the past 9 years, or having had a positive fecal immunochemical (FIT) test result (or immunochemical fecal occult blood test) in the past year or a positive FIT-DNA test result in the past 3 years.

Clinical Performance Measures

The primary and secondary objectives of this study were to determine the sensitivity and specificity of ColoSense using colonoscopy as the reference method. The primary objective of the study was assessed using three co-primary performance metrics:

- Sensitivity for subjects with colorectal cancer (CRC) that were detected as positive by ColoSense.
- Sensitivity for subjects with advanced adenomas (AA) and serrated precancerous lesions (SPL) that were detected as positive by ColoSense.
- Specificity for subjects with Non-advanced Precancerous Lesions (NAPL) or Negative Findings (NEG) that were detected as negative by ColoSense.

Secondary objectives analyzed various subsets of clinical categories. For secondary objectives, sensitivity performance metrics included: sensitivity for various stages of colorectal cancer (CRC) and sensitivity for various subtypes of advanced adenomas (AA). For secondary objectives, specificity performance metrics included: specificity for non-advanced precancerous lesions (NAPL), negative findings (NEG), and no lesions on colonoscopy.

Study Subjects

In total, 8,289 subjects completed all study requirements within study inclusion / exclusion criteria. Within these 8,289 subjects, there were 526 subjects that had self-reported first-degree relative(s) with CRC. As such, these individuals are not included in the primary effectiveness population (Average-risk Cohort) which consisted of 7,763 subjects at Average-risk for CRC. Primary endpoints were assessed for the Average-risk Cohort in the CRC-PREVENT clinical trial. The 526 subjects that had first-degree relative(s) with CRC were analyzed as a separate subgroup (Non-Average-risk Cohort), and results from these subjects are excluded from the primary performance analysis.

Study Population and Baseline Demographics

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. The 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. Across all subjects, 29.0% of patients had an income below \$50,000 per year and 28.3% were on public insurance or had no insurance. Additionally, 33.5% of subjects had prior tobacco exposure.

Table 17. Baseline demographics for the Average-risk Cohort

Category	Subgroup	Primary Effectiveness Population
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		(n = 7,763)
Age	Mean (range)	55.3 (45 years to 90 years)
	45-49	1,820 (23.4%)
	50-54	2,598 (33.5%)
	55-59	976 (12.6%)
	60-64	1,231 (15.9%)
	>65	1,138 (14.7%)
Sex	Female	4,643 (59.8%)
	Male	3,111 (40.1%)
	Other	9 (0.1%)
Race	American Indian / Alaskan Native	64 (0.8%)
	Asian	307 (4.0%)
	Black / African American	821 (10.6%)
	Native Hawaiian / Other Pacific Islander	17 (0.2%)
	White	6,268 (80.7%)
	Other	286 (3.7%)
Ethnicity	Hispanic or Latino	500 (6.4%)
	Not Hispanic or Latino	7,121 (91.7%)
	Prefer not to Answer	142 (21.8%)
Tobacco Exposure	Yes	2,597 (33.5%)
	No	5,166 (66.5%)
Family History of CRC (first-degree relative)	Yes	0 (0.0%)
	No	7,763 (100.0%)

Primary and Secondary Performance Metrics

Table 18 shows the primary performance characteristics of ColoSense. Sensitivity for CRC was 92.6%, sensitivity for AA or SPL was 41.3%, and specificity for NAPL or NEG was 85.6%.

Table 18. Primary performance characteristics for the Average-risk Cohort

Sensitivity (+/n) [95% CI]	Average-risk Cohort (n = 7,763)
Sensitivity (CRC)	92.6% (25 / 27) [76% - 99%]
Sensitivity (AA or SPL)	41.3% (253 / 612) [37% - 45%]
Specificity (-/n) [95% CI]	Average-risk Cohort (n = 7,763)
Specificity (NAPL or NEG)	85.6% (6,097 / 7,124) [85% - 86%]

Secondary performance characteristics for ColoSense, by histopathology categories and by cancer stage are shown in **table 19** and **table 20**. Sensitivity for AA was 44.9% and sensitivity for SPL was 22.4%. Specificity for NAPL was 83.4%, specificity for NEG was 86.7%, and specificity for no lesions on colonoscopy was 87.5%. 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 19. Secondary performance characteristics for the Average-risk Cohort

Sensitivity (+/n) [95% CI]	Average-risk Cohort (n = 7,763)
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Sensitivity (AA)	44.9% (231 / 514) [41% - 49%]
Sensitivity (SPL)	22.4% (22 / 98) [15% - 32%]
Specificity (-/n) [95% CI]	Average-risk Cohort (n = 7,763)
Specificity (NAPL)	83.4% (1,969 / 2,362) [82% - 85%]
Specificity (NEG)	86.7% (4,128 / 4,762) [86% - 88%]
Specificity (no lesions on colonoscopy)	87.5% (2,891 / 3,303) [86% - 89%]

Table 20. ColoSense results by clinical category for the Average-risk Cohort

Category	Average-risk Cohort (n = 7,763)
Positive Findings	Sensitivity (+/n) [95% CI]
Colorectal cancer (CRC)	92.6% (25 / 27) [76%-99%]
Stage I	100.0% (12 / 12) [74%-100%]
Stage II	71.4% (5 / 7) [29%-96%]
Stage III	100.0% (8 / 8) [63%-100%]
Unknown	-
Advanced adenomas (AA)	44.9% (231 / 514) [41%-49%]
High-grade dysplasia or >10 adenomas	64.1% (25 / 39) [47%-79%]
Tubulovillous adenomas, any size	47.4% (65 / 137) [39%-56%]
Tubular adenoma, > 10mm	41.7% (141 / 338) [36%-47%]
Sessile Precancerous Lesions (SPL)	22.4% (22 / 98) [15%-32%]
Negative Findings	Specificity (-/n) [95% CI]
Non-advanced precancerous lesions (NAPL)	83.4% (1,969 / 2,362) [82%-85%]
Negative findings (NEG)	86.7% (4,128 / 4,762) [86%-88%]
Hyperplastic polyps or negative lesions	84.8% (1,237 / 1,459) [83%-87%]
No lesions on colonoscopy	87.5% (2,891 / 3,303) [86%-89%]

Performance by demographic subgroup for the clinical effectiveness population is shown in **table 21**.

Table 21. Primary Performance Characteristics for the Average-risk Cohort

Subgroup	Sensitivity (CRC)	Sensitivity (AA)	Sensitivity (SPL)	Specificity (NAPL)	Specificity (NEG)
Gender					
Female	15 / 17 (88.2%)	93 / 235 (39.6%)	12 / 66 (18.2%)	1,076 / 1,277 (84.3%)	2,656 / 3,048 (87.1%)
Male	10 / 10 (100.0%)	137 / 277 (49.5%)	9 / 31 (29.0%)	891 / 1,083 (82.3%)	1,469 / 1,710 (85.9%)
Other	-	1 / 2 (50.0%)	1 / 1 (100.0%)	2 / 2 (100.0%)	3 / 4 (75.0%)
Age					
45-49	5 / 5 (100.0%)	37 / 84 (44.0%)	3 / 26 (11.5%)	426 / 485 (87.8%)	1,072 / 1,220 (87.9%)
50-54	11 / 11 (100.0%)	68 / 158 (43.0%)	7 / 31 (22.6%)	639 / 767 (83.3%)	1,431 / 1,631 (87.7%)
55-59	1 / 1 (100.0%)	37 / 82 (45.1%)	5 / 15 (33.3%)	249 / 304 (81.9%)	490 / 574 (85.4%)
60-64	6 / 7 (85.7%)	38 / 88 (43.2%)	5 / 17 (29.4%)	303 / 379 (79.9%)	628 / 740 (84.9%)
>65	2 / 3 (66.7%)	51 / 102 (50.0%)	2 / 9 (22.2%)	352 / 427 (82.4%)	507 / 597 (84.9%)
Race					
AI/AN	-	4 / 7 (57.1%)	1 / 2 (50.0%)	21 / 24 (87.5%)	30 / 31 (96.8%)
Asian	-	4 / 9 (44.4%)	0 / 3 (0.0%)	81 / 95 (85.3%)	183 / 200 (91.5%)
Black	3 / 4 (75.0%)	30 / 66 (45.5%)	2 / 4 (50.0%)	173 / 212 (81.6%)	436 / 535 (81.5%)
NH/OPI	-	0 / 1 (0.0%)	-	2 / 3 (66.7%)	12 / 13 (92.3%)
White	22 / 23 (95.7%)	186 / 409 (45.5%)	19 / 86 (22.1%)	1,628 / 1,947 (83.6%)	3,307 / 3,803 (87.0%)
Other	-	3 / 16 (18.8%)	0 / 1 (0.0%)	55 / 71 (77.5%)	133 / 153 (86.9%)
No answer	-	4 / 6 (66.7%)	0 / 2 (0.0%)	9 / 10 (90.0%)	27 / 27 (100.0%)
Ethnicity					
Hispanic	-	14 / 32 (43.8%)	0 / 5 (0.0%)	123 / 150 (82.0%)	267 / 313 (85.3%)
Not Hispanic	25 / 26 (96.2%)	208 / 470 (44.3%)	22 / 92 (23.9%)	1,811 / 2,167 (83.6%)	3,787 / 4,366 (86.7%)
Other	0 / 1 (0.0%)	9 / 12 (75.0%)	0 / 1 (0.0%)	35 / 45 (77.8%)	74 / 83 (89.2%)

Using the data above, 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 ColoSense test in the intended use population are shown in **table 22**.

Table 22. Positive predictive value (PPV) and negative predictive value (NPV) for the Average-risk Cohort

Statistic	Average-risk Cohort (n = 7,763)
PPC (CRC)	1.9% (25 / 1,305) [1% - 3%]
PPV (CRC or AA)	19.6% (256 / 1,305) [17% - 22%]
PPV (CRC, AA, or SPL)	21.3% (278 / 1,305) [19% - 24%]
NPV (NAPL or NEG)	94.4% (6,097 / 6,458) [94% - 95%]

Performance in the Non-Average-risk Cohort

The original clinical study of 8,289 subjects included the Non-Average-risk Cohort consisting of 526 subjects with a first-degree family history of CRC, which were excluded from clinical performance assessments in the average-risk population. Average age for this cohort was 56.4 years (range = 45 years to 80 years) and 58.0% were female (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. Across all subjects, 34.8% of patients had an income below \$50,000 per year and 33.1% were on public insurance or had no insurance. This subgroup analysis was not powered to support a performance for individuals that had first-degree relative(s) with CRC.

This population included 4 CRC patients, 55 subjects with AA, and 7 subjects with SPL. ColoSense results for these 526 subjects are shown in **table 23**.

Table 23. ColoSense results by clinical category for the Non-Average-risk Cohort

Category	Non-Average-risk Cohort (n = 526)
Positive Findings	Sensitivity (+/n) [95% CI]
Colorectal cancer (CRC)	
Stage I	100.0% (1 / 1) [2% - 100%]
Stage II	100.0% (3 / 3) [30% - 100%]
Stage III	-
Unknown	-
Advanced adenomas (AA)	
High-grade dysplasia or >10 adenomas	71.4% (5 / 7) [29% - 96%]
Tubulovillous adenomas, any size	42.9% (6 / 14) [18% - 71%]
Tubular adenoma, > 10mm	47.1% (16 / 34) [30% - 65%]
Sessile Precancerous Lesions (SPL)	28.6% (2 / 7) [4% - 71%]
Negative Findings	Specificity (-/n) [95% CI]
Non-advanced precancerous lesions (NAPL)	79.5% (140 / 176) [73% - 85%]
Negative findings (NEG)	
Hyperplastic polyps or negative lesions	86.6% (246 / 284) [82% - 90%]
No lesions on colonoscopy	78.0% (71 / 91) [68% - 86%]
	90.7% (175 / 193) [86% - 94%]

16. ABBREVIATIONS USED

AA	Advanced Adenoma
ACY1	Aminoacylase 1 Gene
AREG	Amphiregulin Gene
BTC	Blank Template Control
CDH1	Cadherin 1 Gene

CRC	Colorectal Cancer
EGLN2	Egl-9 Family Hypoxia Inducible Factor 2 Gene
FAP	Familial Adenomatous Polyposis
FIT	Fecal Immunochemical Test
FOBT	Fecal occult blood test
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase Gene
IBD	Inflammatory Bowel Disease
iFOBT	Fecal Immunochemical Blood Test
KRAS	KRAS Proto-Oncogene
LIMS	Laboratory Information Management System
NAPL	Non-advanced precancerous lesions
NEG	Negative Findings
NPV	Negative Predictive Value
PPV	Positive Predictive Value
RNA	Ribonucleic Acid
SMAD4	SMAD Family Member 4 Gene
SPL	Sessile Precancerous Lesions
TNFRSF10B	TNF Receptor Superfamily Member 10B

17. KEY SYMBOLS USED

Symbol	Description
	In Vitro Diagnostic Medical Device
	Manufacturer Information
	Temperature Storage Range

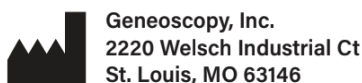
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19. CONTACT INFORMATION

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