cologuard plus™
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Clinician Brochure

Exact Sciences Corporation
5505 Endeavor Lane
Madison, WI 53719 USA

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1 Intended Use and Indications for Use

The Cologuard Plus™ test is a qualitative in vitro diagnostic test intended for the detection of colorectal neoplasia-associated DNA markers and for the presence of occult hemoglobin in human stool. The Cologuard Plus test is performed on samples collected using the Cologuard Plus Collection Kit. A positive result may indicate the presence of colorectal cancer (CRC) or advanced precancerous lesions (APL) and should be followed by colonoscopy. The Cologuard Plus test is indicated to screen adults 45 years or older, who are at average risk for CRC. The Cologuard Plus test is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

The Cologuard Plus test is performed at Exact Sciences, Madison, WI.

2 Contraindications

The Cologuard Plus test is not indicated for use in patients who have the following:

- A personal history of CRC or APLs.
- A positive result from another CRC screening method within the last 6 months, or:
  - 12 months for a fecal occult blood test (FOBT) or a fecal immunochemical test (FIT)
  - 36 months for a FIT-DNA test
- A family history of CRC, defined as having a first-degree relative (parent, sibling, or child) with a CRC diagnosis at any age.
- Personal history of any of the following high-risk conditions for CRC:
  - A diagnosis of Inflammatory Bowel Disease (Chronic Ulcerative Colitis, Crohn's Disease).
  - A diagnosis of a relevant familial (hereditary) cancer syndrome or other polyposis syndrome, including but not limited to: Familial adenomatous polyposis (FAP or Gardner's), Hereditary non-polyposis colorectal cancer syndrome (HNPCC or Lynch), Peutz-Jeghers, MYH-Associated Polyposis (MAP), Turcot's (or Crail's), Cowden's, Juvenile Polyposis, Cronkhite-Canada, Neurofibromatosis, or Serrated Polyposis.

3 Warnings and Precautions

- Patients should not provide a sample if they are experiencing diarrhea or have known blood in their urine or stool (e.g., from bleeding hemorrhoids, bleeding cuts or wounds on their hands, rectal bleeding, or menstrual bleeding). Unexpected bleeding should be discussed with your healthcare provider.
- Reference national guidelines for the recommended screening ages for colorectal cancer.[4] The decision to screen persons over the age of 75 should be made on an individualized basis in consultation with your healthcare provider. Cologuard Plus test results should be interpreted with caution in older patients as the rate of false positive results increases with age.
- The Cologuard Plus test may produce false negative or false positive results. A false positive result occurs when the Cologuard Plus test produces a positive result, even though a colonoscopy will not find CRC or APL. A false negative result occurs when the Cologuard Plus test does not detect an APL or CRC even when a colonoscopy identifies either of these findings.
  - Out of every 100 patients testing positive, approximately 3 patients will have CRC, 34 patients will have APL, 33 will have a non-advanced adenoma, and 30 will have no neoplastic findings.
  - Out of every 10,000 patients testing negative, approximately 2 will be falsely assured that they do not have CRC. Out of every 100 patients testing negative, approximately 7 patients will be falsely assured that they do not have APL.
- A negative Cologuard Plus test result does not guarantee the absence of CRC or APL. Patients with a negative Cologuard Plus test result should continue participating in colorectal cancer screening programs, at the appropriate guideline recommended intervals.
- The performance of the Cologuard Plus test has been established in a cross-sectional study (i.e., single point in time). Programmatic performance of the Cologuard Plus test (i.e., benefits and risks with repeated testing over an established period of time) has not been studied. Non-inferiority or superiority of the Cologuard Plus test's programmatic sensitivity as compared to other recommended screening methods for CRC and APL has not been established.
- To ensure the integrity of the sample, the laboratory must receive the patient specimens within 144 hours of collection. Patients should send stool samples to the laboratory according to the instructions included in the Cologuard Plus Collection Kit.
- Patients should be advised of the caution listed in the Cologuard Plus Collection Kit instructions. Patients should NOT drink the preservative liquid.
- The risks related to using the Cologuard Plus Collection Kit are low, with no serious adverse events reported among people in a clinical trial. Patients

should be careful when opening and closing the lids to avoid the risk of hand strain. Fecal samples should be treated as if they are potentially infectious.

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4 The Cologuard Plus Test Overview

4.1 Summary of the Cologuard Plus Test Performance

The Cologuard Plus test demonstrated 95.3% CRC sensitivity, 43.3% APL sensitivity, and 90.7% specificity among participants with neither CRC nor APL.

4.2 Patient Samples for the Cologuard Plus Test

Patients are not required to undergo bowel preparation or follow dietary or medication restrictions in order to complete the test. Patients follow the detailed instructions received with the Cologuard Plus Collection Kit, consisting of a container for collection of stool for DNA testing and a separate Tube and Probe for collection of stool for hemoglobin testing. Both of these stool samples are required to obtain a Cologuard Plus test result. Samples are sent to Exact Sciences Laboratories for processing and testing.

4.3 The Cologuard Plus Test Patient Navigation Program

The Cologuard Plus test includes a patient support program. Customer Care Specialists are available 24 hours a day, 7 days a week to communicate with patients in over 240 languages about the Cologuard Plus test sample collection or return questions. Representatives are also available to answer billing or reimbursement questions. Exact Sciences Laboratories sends patients reminders about completing the Cologuard Plus Collection Kit. This program also provides tracking for healthcare providers so they can measure and monitor patient adherence to Cologuard Plus test screening.

5 Colorectal Cancer Overview

Colorectal cancer (CRC) is the second leading cause of cancer death among men and women in the United States, with more than 153,000 individuals diagnosed annually.[1] One in 24 Americans will suffer from CRC during their lifetime.[10] Early detection by screening has been shown to reduce CRC mortality.[2-8] Based on increasing incidence of CRC in younger adults, current guidelines for CRC screening in the average-risk population recommend initiation of screening at age 45.[3-6,] The 2021 US Preventive Services Task Force (USPSTF) recommendation concludes that initiating colorectal cancer screening at age 45 provides moderate certainty of moderate net benefit,[4] whereas the 2018 guideline update from the American Cancer Society (ACS) gave a qualified recommendation to initiate screening at age 45 in average risk individuals.[3] In addition, the American College of Gastroenterology (ACG) and US Multi-Society Task Force (MSTF) updated their CRC screening guidelines in 2021 to recommend initiation of screening at age 45 for average risk individuals.[5,6]

Approximately 40% of adults 45 years of age or older are not current with recommended CRC screening.[1] Less than half of adults 50-54 years of age and only 17.8% of adults ages 40-49 report recent screening for CRC.[3]

Detection of potentially pre-malignant lesions, also known as advanced precancerous lesions, is essential for CRC prevention. APLs include any size adenomas with carcinoma in situ or high-grade dysplasia (HGD), adenomas with villous growth patterns (≥25%), adenomas ≥1.0 cm in size or serrated lesions ≥1.0 cm in size.[7-9] Serrated lesions (polyps and sessile serrated adenomas) are typically found in the proximal colon, occur more frequently in the elderly, are often flat and inconspicuous endoscopically, and may have a more aggressive natural history than classic colorectal adenomas.[9]

6 Device Description

The Cologuard Plus test is an in vitro diagnostic device designed to analyze a patient's stool for the presence of DNA and hemoglobin markers which may indicate the presence of CRC or APL. Specifically, two independent categories of biomarkers are targeted and provide an additive association for the detection of CRC and pre-malignant neoplasms. The combined result/composite score gives a qualitative result, Positive (abnormal) or Negative (normal) which is associated with increased or decreased likelihood of CRC and APL, respectively.

The first category of biomarkers detects epigenetic DNA changes characterized by aberrant gene promoter region methylation. The specific methylated gene targets include ceramide synthase 4 gene *(LASS4)*, leucine-rich repeat-containing protein 4 gene *(LRRC4)*, and protein phosphatase 2 regulatory subunit B' gene *(PPP2R5C)*. *LASS4, LRRC4,* and *PPP2R5C* have been shown to be hypermethylated in colorectal cancer.[12-14] The Cologuard Plus procedure incorporates bisulfite conversion of non-methylated cytosine residues to uracil

in the DNA sequence to enable sensitive detection of hypermethylated *LASS4, LRRC4, and PPP2R5C*. The second category of biomarker is non-DNA based and detects hemoglobin, which can be associated with colonic bleeding. Results from the molecular and hemoglobin assays are integrated by the laboratory analysis to determine a Positive or Negative reportable result or No Result Obtained.[16]

6.1 Assay Technology

The patient stool samples are processed at Exact Sciences Laboratories to isolate the DNA for testing. Amplification and detection of the hypermethylated target DNA *LASS4*, *LRRC4*, *PPP2R5C*, and *ZDHHC1* (a reference gene) is performed by incorporating bisulfite conversion of non-methylated cytosine residues to uracil in the DNA sequence to enable sensitive detection of the hypermethylated target DNA using the Long-probe Quantitative Amplified Signal (LQAS) technology, which combines real-time PCR and invasive cleavage to perform allele-specific amplification and detection of methylated target DNA in the molecular assay. In a parallel workflow, the hemoglobin stool sample is prepared and analyzed in a quantitative Enzyme-Linked Immunosorbent Assay (ELISA) that determines the concentration of hemoglobin in the sample.

Run control samples for both the DNA assay and hemoglobin assay are tested along with patient samples to show that the process has been performed appropriately. Results from the DNA and hemoglobin assays are integrated during analysis to determine a Positive or Negative reportable result or No Result Obtained.

7 Clinical Study: Multi-Target Stool DNA Test for Colorectal Cancer Screening: BLUE-C

7.1 Overview

The Cologuard Plus test was the subject of a prospective, cross-sectional, multi-center, pivotal trial, Multi-Target Stool DNA Test for Colorectal Cancer Screening: BLUE-C Study, ("BLUE-C" or "the study").[17] A total of 26,758 participants were enrolled from 186 sites, including both colonoscopy centers and primary care sites, in the United States. The results of the study demonstrated the safety and effectiveness of the Cologuard Plus test as a screening test for the detection of markers associated with the presence of CRC and APL. The Cologuard Plus test demonstrated 95.3% CRC sensitivity and 90.7% specificity (specificity in this study excludes CRC and APL), using colonoscopy with histopathological confirmation as the reference method. These results met the protocol-specified criteria for primary performance measures and study success. The study further compared CRC and APL detection by the Cologuard Plus test to a commercially available fecal immunochemical test (OC-Auto® Micro 80, Polymedco, Inc.) ("FIT"), demonstrating superiority for CRC ($p<0.0001$) and APL ($p<0.0001$) sensitivity.

7.2 Study Design

The study was designed to enroll male or female participants, who were at average risk for development of CRC and asymptomatic for gastrointestinal symptoms warranting diagnostic colonoscopy. Subject enrollment was enriched toward a slightly older population to increase the point prevalence of colorectal cancer in this study. Enrollment was also focused on colonoscopy-naïve participants aged ≥55 years because of the higher prevalence of CRC in this population. Forty-eight percent of participants in the actual study population were 65 years of age or older.

In addition to the study enrollment eligibility criteria, the primary analysis population excluded participants with a first-degree relative with CRC at any age, as well as participants under the age of 45 years.

Trial participants provided a stool sample and subsequently underwent colonoscopy within 180 days of stool collection. Participants collected stool samples for the Cologuard Plus test and independent FIT testing at home. Participants then underwent colonoscopy per standard of care. Participants and physicians remained blinded to the results of the Cologuard Plus test and the FIT. Results from the Cologuard Plus test and FIT were compared to the results of the colonoscopy examination and histopathologic diagnosis of all significant lesions either biopsied or removed.

Histopathological results from biopsied tissue or excised lesions were categorized based on the most clinically significant lesion present (i.e. the index lesion) by a central pathologist according to the pre- specified standards outlined in Table 7-1. Participants with no findings on colonoscopy and no biopsy(ies) taken were categorized as 6.2. Sensitivity calculations were performed using positive findings in categories 1 and 2 while specificity was calculated using categories 3 through 6 (all findings excluding CRC and APL). Stages of CRC were recorded based on the American Joint Committee on Cancer (AJCC) Staging System, 8th edition.[15]

Table 7-1: Participant Categorization Based on Histopathologic Diagnosis of the Index Lesion

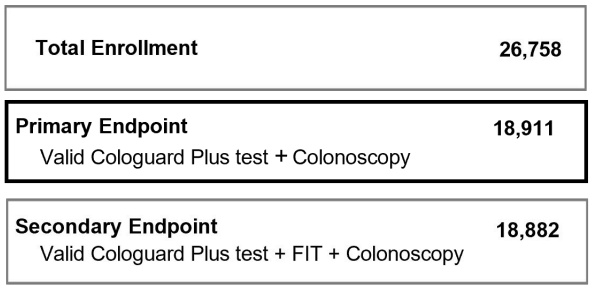
Category	Description
1	Stage I-IV colorectal cancer, any size
2	Advanced Precancerous Lesions (APL), including the following subcategories:
2.1	High-grade dysplasia or ≥10 adenomas, any size
2.1a	High-grade dysplasia, any size
2.1b	≥10 adenomas, any size
2.2	Tubulovillous adenoma, any size
2.3	Tubular adenoma, ≥10 mm
2.4	Sessile serrated lesion with dysplasia (SSLD); Traditional serrated adenoma (TSA), Conventional adenoma with serrated architecture; Sessile serrated lesion; ≥10 mm
3	3-9 adenomas or sessile serrated lesions, <10 mm, non-advanced
4	1-2 adenomas or sessile serrated lesions, 5-9 mm, non-advanced
5	1-2 adenomas or sessile serrated lesions, <5 mm, non-advanced
6	Negative: no adenocarcinoma of the colorectum, no adenomas or SSA/SSP
6.1	Hyperplastic polyps or non-neoplastic lesions
6.2	No lesions on colonoscopy
X	Index Lesion could not be categorized because tissue/report was lost/not provided or histopathological diagnosis could not be determined.

7.3 Demographic and Baseline Characteristics

Study enrollment and population demographics are summarized in Figure 7-1.

Of the total 26,758 participants enrolled in the study, 18,911 participants with colonoscopy and Cologuard Plus test data were included in the primary analysis population. This population includes 85 participants with CRC. Analyses conducted to assess the impact of enrichment strategies and evaluate potential bias associated with participants excluded from the analysis population yielded results consistent with the primary analyses.

The average age of participants was 63.0 years, and there was a slightly higher percentage of female participants 53.1% (10,035/18,911) as compared with male participants 46.9% (8,876/18,911). The majority of participants were White, not Hispanic or Latino 59.70% (11,286/18,911). Among the minorities in the study population, there were Hispanic or Latino 16.4% (3,094/18,911); Black or African American, not Hispanic or Latino 13.4% (2,532/18,911); and Asian, not Hispanic or Latino 9.0% (1,704/18,911). Other race and ethnicity categories with smaller enrollment distribution are included in the figure below. Average Body Mass Index (BMI) was 29.5 kg/m^2 and the majority of participants never smoked 63.6% (12,019/18,911).



Demographics		
Age (Years)	Average	63.0
	Range	45-86
Sex	Male	46.9%
	Female	53.1%
BMI (kg/m²)	Average	29.5
	Range	13.0-69.2
Ethnicity/ Race	Hispanic/Latino	16.4%
	Not Hispanic or Latino	
	White	59.7%
	Black	13.4%
	Asian	9.0%
	Amer. Ind/Alaskan Native	0.4%
	Native Hawaiian or Other Pacific Islander	0.1%
	Multiracial	0.4%
	Other	0.6%
Smoking History	Never Smoked	63.6%
	Former Smoker	24.4%
	Smoker	12.1%

Figure 7-1: Clinical Study Demographics

7.4 Clinical Performance Measures

The primary and secondary performance measures for the clinical study are summarized in Table 7-2 below. The primary performance measures were the sensitivity of the Cologuard Plus test for CRC and specificity among participants not having CRC or APL, using colonoscopy with histopathology as the reference method. The primary analysis required the one-sided 97.5% exact confidence interval (CI) to be greater than the 75% null hypothesis value for the sensitivity of the Cologuard Plus test for CRC. The specificity analysis required the one-sided 97.5% exact CI to be greater than the 85.9% null hypothesis value.

For the secondary performance measures, evaluating the Cologuard Plus test sensitivity for APL detection required the one-sided 97.5% exact CI to exceed the null hypothesis value of 38.9%; evaluating specificity of the Cologuard Plus test for no colorectal neoplastic findings required the one- sided 97.5% exact CI to exceed the null hypothesis value of 87.5%. The head-to-head comparisons with FIT were performed using exact McNemar's tests for paired proportions at the one-sided 2.5% significance level.

Table 7-2: Clinical Study Primary and Secondary Performance Measures

Primary Performance Measures	• Determine the sensitivity of the Cologuard Plus test for CRC detection. • Determine the specificity of the Cologuard Plus test for Categories 3–6 (no APL or CRC).
Secondary Performance Measures	• Determine the sensitivity of the Cologuard Plus test for APL detection. • Compare the Cologuard Plus test sensitivities for CRC and APL detection to FIT. • Evaluate the specificity of the Cologuard Plus test for participants with no colorectal neoplastic findings (Category 6).

7.5 Summary of Clinical Study Results

Results from the clinical study, summarized in Table 7-3, demonstrated that the Cologuard Plus test successfully met both the primary and secondary performance measures of the study, establishing a clinically meaningful sensitivity for CRC and specificity. Sensitivity of the Cologuard Plus test for CRC was 95.3% (81/85) with a one-sided 97.5% lower confidence bound of 88.4%, above the pre-specified threshold for study success. Sensitivity of the Cologuard Plus test for APL detection was 43.3%, with a one-sided 97.5% lower confidence bound of 41.1%, exceeding the protocol-specified threshold for study success. In addition, the Cologuard Plus test successfully demonstrated a

clinically meaningful specificity according to the protocol-specified criteria. The specificity of the Cologuard Plus test for Category 3–6 was 90.7%, with a one-sided 97.5% lower confidence bound of 90.3%, above the prespecified threshold for study success. For Category 6, the specificity of the Cologuard Plus test was 92.7% with a one- sided 97.5% lower confidence bound of 92.2%, above the pre-specified threshold for study success.

Table 7-3: Summary of the Cologuard Plus Test Performance

Colonoscopy/Histo-pathology	Primary Effectiveness Population
	Sensitivity %, (95% CI) (n detected/N)
CRC	95.3 (88.4, 98.7) (81/85)
APL	43.3 (41.1, 45.5) (849/1,962)
	Specificity % (95% CI) (n negative/N)
Category 3–6	90.7 (90.3, 91.1) (15,297/16,864)
No colorectal neoplasia (Category 6)	92.7 (92.2, 93.2) (9,609/10,361)

The clinical study also compared the Cologuard Plus test sensitivity and specificity to an independent commercially available FIT. Results demonstrated superiority of the Cologuard Plus test to FIT for sensitivity in detecting CRC. Sensitivity for CRC was greater for the Cologuard Plus test compared to FIT (95.3% vs. 70.6%, respectively, exact McNemar p<0.0001). The Cologuard Plus test identified 21 of 25 (84.0%) CRC cases that were missed by FIT, while FIT did not identify any cancer cases that were not identified by the Cologuard Plus test.

Sensitivity for APL was greater for the Cologuard Plus test compared to FIT (43.3% vs. 23.3%, respectively, exact McNemar p<0.0001). The Cologuard Plus test identified 506 of 1,503 (33.7%) APL cases missed by FIT, while FIT identified 115 of 1,112 (10.3%) APL cases missed by the Cologuard Plus test.

The specificity for Category 3–6 of the Cologuard Plus test was 90.7% and of FIT was 94.8%. These specificity measures excluded CRC and APL for both tests.

The positive and negative predictive values (PPV and NPV) of the Cologuard Plus test were calculated. The PPV of the Cologuard Plus test was 3.2% (81/2,497) for CRC and 34.0% (849/2,497) for APL. Among the participants with a positive Cologuard Plus test result, 69.9% (1,745/2,497) were found to have a CRC, APL, or non-advanced adenoma. The NPV of the Cologuard Plus test was 99.98% (16,410/16,414) for CRC, and 93.2% (15,297/16,414) for advanced neoplasia (CRC or APL). Clinical results show that a negative patient result for the Cologuard Plus test gives 99.98% assurance that the patient does not have CRC and a 93.2% chance that the patient does not have any CRC or APL.

Table 7-4: Positive and Negative Predictive Values: Index Lesion Categorization by the Cologuard Plus Test Result

Index Lesion Categorization	Positive Predictive Value (PPV), % (95% CI); n/N positive test results	1-Negative Predictive Value (1-NPV), % (95% CI); n/N negative test results
CRC (n=85)	3.2 (2.6-4.0); 81/2,497	0.02 (0.01-0.06); 4/16,414
APL (n=1,962)	34.0 (32.1-35.9); 849/2,497	6.8 (6.4-7.2); 1,113/16,414
Category 3–5 (n=6,503)	32.6 (30.8-34.5); 815/2,497	34.7 (33.9-35.4); 5,688/16,414
Category 6 (n=10,361)	30.1 (28.3-32.0); 752/2,497	58.5 (57.8-59.3); 9,609/16,414

Age-weighted to the U.S. Population, Category 3-6 specificity was 91.8% (95% CI 91.2-92.4) and Category 6 specificity was 93.8% (95% CI 93.2-94.5).

7.6 The Cologuard Plus Test and FIT Sensitivity by Lesion Subgroups

The Cologuard Plus test demonstrated high sensitivity for detection of lesions and polyps which historically have been difficult to capture with FIT, including early-stage CRC, proximal lesions, and higher risk precancerous lesions. The Cologuard Plus test demonstrated a numerically greater sensitivity than FIT for detection of CRC and APLs across lesion subgroups. Sensitivity results are summarized in Table 7-5 and Table 7-6.

The CRC sensitivity of the Cologuard Plus across all cancer stages was as follows: 88.0% (22/25) in Stage I, 92.9% (13/14) in Stage II, 100% (30/30) in Stage III, and 100.0% (12/12) in Stage IV (See Table 7-5). In the curative stages, Stages I–III combined, the Cologuard Plus test sensitivity was 94.2% (65/69). CRC sensitivity for independent FIT was substantially lower at 56.0% (14/25) in Stage I, 78.6% (11/14) in Stage II, 73.3%

(22/30) in Stage III, 83.3% (10/12) in Stage IV, and 68.1% (47/69) in Stage I–III combined.

The CRC sensitivity of the Cologuard Plus test was generally consistent across lesion sizes and locations: 87.5% (7/8) in CRCs 10–19 mm, 92.3% (12/13) in CRCs 20– 29mm, 96.8% (60/62) in CRCs ≥30 mm, 93.5% (29/31) in proximal CRCs, 93.8% (30/32) in distal CRCs, and 100% (22/22) in rectal cancers. The CRC sensitivity of the independent FIT was substantially lower: 62.5% (5/8) in CRCs 10-19 mm, 61.5% (8/13) in CRCs 20-29 mm, 72.6% (45/62) in CRCs ≥30 mm, 61.3% (19/31) in proximal CRCs, 78.1% (25/32) in distal CRCs, and 72.7% (16/22) in rectal cancers.

APL sensitivity of the Cologuard Plus test, delineated in Table 7-6, increased with lesion size, from 33.3% (2/6) in APLs <5 mm, 28.2% (20/71) in APLs 5-9 mm, 39% (609/1,561) in APLs 10-19 mm, 62.6% (139/222) in APLs 20-29 mm, and 78.0% (78/100) in APLs ≥30 mm. The independent FIT APL sensitivity was substantially lower for each size and location, at 0% (0/6) in APLs <5 mm, 26.8% (19/71) in APLs 5-9 mm, 20.5% (320/1,561) in APLs 10-19 mm, 32.4% (72/222) in APLs 20-29 mm, and 46.0% (46/100) in APLs ≥ 30mm. APL sensitivity of the Cologuard Plus test by location was 39.3% (440/1,120) in proximal APLs, 48.0% (315/656) in distal APLs, and 50.5% (93/184) in rectal APLs. FIT APL sensitivity by location was also substantially lower: 15.7% (176/1,120) in proximal APLs, 35.7% (234/656) in distal APLs, and 25.5% (47/184) in rectal APLs.

Cologuard Plus test APL sensitivity was 73.6% (78/106) for high grade dysplasia, 54.8% (269/491) for tubulovillous adenomas of any size, 33.3% (359/1,077) for tubular adenomas ≥10 mm, and 49.4% (116/235) for serrated lesions≥10 mm. In the higher risk APL combination of all high-grade dysplasia plus any APL ≥15 mm or ≥20 mm, Cologuard Plus test sensitivity was 59.5% (433/728) and 64.7% (275/425) respectively.

FIT sensitivity was 48.1% (51/106) for high grade dysplasia, 33.2% (163/491) for tubulovillous adenomas of any size, 19.5% (210/1,077) for tubular adenomas ≥10 mm, and 4.7% (11/235) for serrated lesions ≥10 mm. In the higher risk APL combination of all high-grade dysplasia plus any APL ≥15 mm or ≥20 mm, independent FIT sensitivity was 32.3% (235/728) and 38.1% (162/425) respectively. As a preplanned exploratory analysis, APL sensitivity was compared between the Cologuard Plus test and FIT for each APL subcategory; for each subcategory, the Cologuard Plus test sensitivity was superior to FIT ($p<0.0001$ for each by McNemar test).

Table 7-5: The Cologuard Plus test CRC Sensitivity by Colonoscopy Categories, Compared to Independent FIT CRC Sensitivity

Subgroup	Cologuard Plus n/N	Cologuard Plus Sensitivity	FIT n/N	FIT Sensitivity
Cancer Stage[15]				
I	22/25	88.0%	14/25	56.0%
II	13/14	92.9%	11/14	78.6%
III	30/30	100.0%	22/30	73.3%
IV	12/12	100.0%	10/12	83.3%
X	4/4	100.0%	3/4	75.0%
Stage I–III combined	65/69	94.2%	47/69	68.1%
Cancer Size				
<5 mm	1/1	100.0%	1/1	100.0%
5–9 mm	1/1	100.0%	1/1	100.0%
10–19 mm	7/8	87.5%	5/8	62.5%
20–29 mm	12/13	92.3%	8/13	61.5%
≥30 mm	60/62	96.8%	45/62	72.6%
Cancer Location				
Proximal	29/31	93.5%	19/31	61.3%
Distal	30/32	93.8%	25/32	78.1%

Subgroup	Cologuard Plus n/N	Cologuard Plus Sensitivity	FIT n/N	FIT Sensitivity
Rectal	22/22	100.0%	16/22	72.7%

Table 7-6: The Cologuard Plus test APL Sensitivity by Colonoscopy Categories, Compared to Independent FIT APL Sensitivity

APL Subgroup	Cologuard Plus n/N	Cologuard Plus Sensitivity	FIT n/N	FIT Sensitivity
APL Subtype[a]				
High-grade dysplasia or ≥10 adenomas, any size	104/157	66.2%	73/157	46.5%
High-grade dysplasia, any size	78/106	73.6%	51/106	48.1%
≥10 adenomas, any size	26/51	51.0%	22/51	43.1%
Tubulovillous adenoma, any size	269/491	54.8%	163/491	33.2%
Tubular adenoma≥10 mm	359/1,077	33.3%	210/1,077	19.5%
Sessile serrated lesion with dysplasia (SSLD); Traditional serrated adenoma (TSA), Conventional adenoma with serrated architecture; Sessile serrated lesion; ≥10 mm	116/235	49.4%	11/235	4.7%
APL Location				
Proximal	440/1,120	39.3%	176/1,120	15.7%
Distal	315/656	48.0%	234/656	35.7%
Rectal	93/184	50.5%	47/184	25.5%
Lesion Size				
<5 mm	2/6	33.3%	0/6	0.0%
5–9 mm	20/71	28.2%	19/71	26.8%
10–19 mm	609/1,561	39.0%	320/1,561	20.5%
20–19 mm	139/222	62.6%	72/222	32.4%
≥30 mm	78/100	78.0%	46/100	46.0%
All High-Grade Dysplasia plus any APL				
≥15 mm	433/728	59.5%	235/728	32.3%
≥20 mm	275/425	64.7%	162/425	38.1%

[a] Please refer to Table 7-1: Participant Categorization Based on Histopathologic Diagnosis of the Index Lesion for descriptions of the APL subcategories.

7.7 The Cologuard Plus Test Subgroup Analysis

Please note that the clinical study was not designed or powered to evaluate subgroups and subgroup analyses should be interpreted with that in mind. The clinical study results summarized in Table 7-7 below were analyzed across demographic subgroups. CRC sensitivity was greater than 90% for each age range; sex, at 95.5% (42/44) in males and 95.1% (39/41) in females; and race/ethnicity, at 94.7% (54/57) in White, not Hispanic or Latino 100% (11/11) in Hispanic or Latino, 90.9% (10/11) in Black, not Hispanic or Latino, and 100% (4/4) in Asian participants.

APL sensitivity increased with age, from 28.6% (4/14) for ages 45-49, 32.5% (37/114) for ages 50-54, 41.3% (181/438) for ages 55-59, 39.0% (150/385) for ages 60-64, 46.4% (289/623) for ages 65-69, 47.9% (134/280) for ages 70-74, and 50.0% (54/108) for ages greater than 75. APL sensitivity was 44.1% (494/1,121) in males and 42.2% (355/841) in females, and 46.4% (597/1,287) in White, not Hispanic or Latino, 43.1% (125/290) in Hispanic or Latino, 38.0% (98/258) in Black, not Hispanic or Latino, and 20.0% (20/100) in Asian participants.

Specificity for Category 3–6 of the Cologuard Plus test was high in the younger age groups and remained above 90% through age 64. Specificity was 97.8% (268/274) in participants aged 45-49 years, 96.1% (1,363/1,419) in ages 50-54, 87.4% (1,924/2,201) in ages 70-74, and 85.5% (762/891) in age 75 and older. By sex, specificity was 89.8% (6,928/7,711) in males and 91.4% (8,369/9,153) in females. Specificity of the Cologuard Plus test was 88.9% (8,842/9,942) in non-Hispanic or Latino White, 92.8% (2,593/2,793) in Hispanic or Latino, 92.3% (2,089/2,263) in non-Hispanic or Latino Black, and 95.1% (1,522/1,600) in Asian participants.

Table 7-7: The Cologuard Plus Test Performance by Subgroup

Subgroup	CRC Sensitivity %; n/N	APL Sensitivity %; n/N	Specificity for Category 3-6 %; n/N
Sex			
Male	95.5%; 42/44	44.1%; 494/1,121	89.8%; 6,928/7,711
Female	95.1%; 39/41	42.2%; 355/841	91.4%; 8,369/9,153
Age			
45–49 years	100.0%; 1/1	28.6%; 4/14	97.8%; 268/274
50–54 years	100.0%; 2/2	32.5%; 37/114	96.1%; 1,363/1,419
55–59 years	100.0%; 17/17	41.3%; 181/438	92.5%; 3,788/4,095
60–64 years	94.4%; 17/18	39.0%; 150/385	91.1%; 2,867/3,148
65–69 years	93.1%; 27/29	46.4%; 289/623	89.4%; 4,325/4,836
70–74 years	92.3%; 12/13	47.9%; 134/280	87.4%; 1,924/2,201
≥75 years	100.0%; 5/5	50.0%; 54/108	85.5%; 762/891
Race/Ethnicity			
White, Not Hispanic or Latino	94.7%; 54/57	46.4%; 597/1,287	88.9%; 8,842/9,942
Hispanic or Latino	100.0 %; 11/11	43.1%; 125/290	92.8%; 2,593/2,793
Black or African American, Not Hispanic or Latino	90.9%; 10/11	38.0%; 98/258	92.3%; 2,089/2,263
Asian, Not Hispanic or Latino	100.0%; 4/4	20.0%; 20/100	95.1%; 1,522/1,600
American Indian or Alaskan Native, Not Hispanic or Latino	--------	42.9%; 3/7	90.0%; 54/60
Native Hawaiian or Other Pacific Islander, Not Hispanic or Latino	--------	25.0%; 1/4	94.7%; 18/19
Multiracial, Not Hispanic or Latino	--------	25.0%; 1/4	95.9%; 70/73
Other, Not Hispanic or Latino	100.0%; 2/2	33.3%; 3/9	96.2%; 101/105

7.8 Cross-Reactivity

The potential for cross-reactivity with non-colorectal cancers and inflammatory conditions was evaluated using sample specimens collected from subjects with 12 cancer and disease groups other than colorectal cancer (CRC). The table below indicates the final number of cancer or disease patient samples that were tested.

The false positive fraction (FPF) of test results was calculated as a point estimate and a two-sided 95% confidence interval for each disease group. Each FPF was compared to the estimated FPF for the general intended use (IU) population. The disease groups of lung cancer, esophageal cancer, and inflammatory bowel disease did not overlap the estimated FPF for the general IU population. The other nine groups had observed positive test results rates that are consistent with the FPF for the overall assay.

For the assay specificity analysis, the total number of positive calls per 10,000 patients was estimated to be 8.1 to 9.0 with the inclusion of IBD and 7.7 to 8.0 without, as shown in the following table. This was considered a negligible effect on the Cologuard Plus test positivity.

Table 7-8: Cancers and Diseases Tested for Cross-Reactivity

No.	Cancer or Disease[a]	No. of Valid Samples Tested	Incidence per 10,000 population[b]	% Positivity of Cologuard Plus Result	No. Positive Cologuard Plus Calls in 10,000 Patients
1	Autoimmune Disease[c] (individual disease not specified)	29	3.2–5.4	13.8	0.4–0.7
2	Bladder Cancer	5	1.8	20.0	0.4
3	Breast Cancer	35	12.6	11.4	1.4
4	Esophageal Cancer	11	0.4	36.4	0.1
5	Gynecologic Cancer (i.e., endometrial cancer, vulvar melanoma, and ovarian cancer	41	3.8	4.9	0.2
6	Hepatic Cancer (i.e., liver and bile duct cancer)	5	0.9	20.0	0.2
7	Inflammatory Bowel Disease[c]	30	1.5–3.9	26.7	0.4–1.0
8	Kidney/Renal Pelvis Cancer	20	1.7	10.0	0.2
9	Lung Cancer	30	5.0	33.3	1.7
10	Pancreatic Cancer	13	1.3	15.4	0.2
11	Prostate Cancer	35	11.3	22.9	2.6
12	Stomach Cancer	5	0.7	40.0	0.3
Total (with IBD)					8.1–9.0
Total (without IBD)					7.7–8.0

a USA population-based cancer incidence data were obtained from registries that participate in the CDC's National Program of Cancer Registries and/or the NCI's Surveillance, Epidemiology, and End Results (SEER) Program.

b Cancer prevalence or incidence per 10,000 population was calculated with the assumption the population consists of 50/50 male-to-female.

c Incidence of autoimmune diseases reported for North America include Multiple Sclerosis, Type I Diabetes, Primary Biliary Cirrhosis, Autoimmune Hepatitis, Graves' Disease, Coeliac Disease, Addison's Disease, Sjogren's Syndrome, Systemic Lupus Erythematosus and Rheumatoid Arthritis. See Wang, L., Wang, F., and Gershwin, M.E. (2015). Human autoimmune diseases: a comprehensive update. Journal of Internal Medicine, Volume 278, Issue 4, Pages 369-395.[18]

7.9 Interfering Substances

There are no known interfering substances with the Cologuard Plus test. The Molecular and Hemoglobin assays of the test were challenged independently with the 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, anti-fungal medications, pain relievers, decongestants, stool softeners, urine, alcohol, Vitamin C, iron, common vegetables and fruits, fats, hypomethylation agents, and lipids. There was no observed interference with any substance in either assay. The hemoglobin assay was also tested with antibiotics, anti-inflammatories, anti-fungal drugs, pain relievers, and decongestants with no observed interference. The assays were tested with animal genomic DNA, hemoglobin, and/or myoglobin from commonly eaten animals with no observed interference.

8 Ordering the Cologuard Plus Test

The Cologuard Plus test is available for clinicians to order through Electronic Health Records (EHR) integrated ordering and resulting. Additional ordering options (e.g., EpicCare Link, efax and paper order forms) can be accessed from Exact Sciences Laboratories at http://www.cologuardplus.com.

The Cologuard Plus test includes a patient navigation program that provides attentive service to physicians and patients with live specialists. For any questions about the Cologuard Plus test or specific questions on how to order the test, please contact Exact Sciences Laboratories.

Exact Sciences Laboratories
145 E. Badger Rd, Suite 100
Madison, WI 53713
USA
844-870-8870

8.1 Sample Collection

- Samples for use with the Cologuard Plus test must be collected with the Cologuard Plus Collection Kit, including a stool sample for DNA testing (Container) and a stool sample for Hemoglobin testing (Tube and Probe).
- Patients should not provide a sample if they have diarrhea or blood in their urine or stool from bleeding hemorrhoids, bleeding cuts or wounds on their hands, rectal bleeding, or menstrual bleeding.
- Patients should familiarize themselves with detailed information contained in the Cologuard Plus Patient Guide and collection instructions before completing sample collection.
- The use of this kit requires sitting down on the toilet and standing up from the toilet. Patients should have someone available to help them sit down or stand up if needed.
- To ensure the integrity of the sample, the laboratory must receive patient specimens within 144 hours of collection. Patients should send stool samples to the laboratory using UPS™ according to the instructions included in the Cologuard Plus Collection Kit.

8.2 Instructions for Sample Collection

Once the Cologuard Plus test has been ordered, the patient will receive a Cologuard Plus Collection Kit. Detailed instructions for patient specimen collection are provided in the instructions accompanying the Cologuard Plus Collection Kit. Full closure of the stool collection container should be emphasized to patients to ensure receipt of a usable sample for testing. A toll-free number is also provided with the patient guide to ensure that any patient questions are addressed. An overview of the 7 steps in the collection process is provided in the following figure.

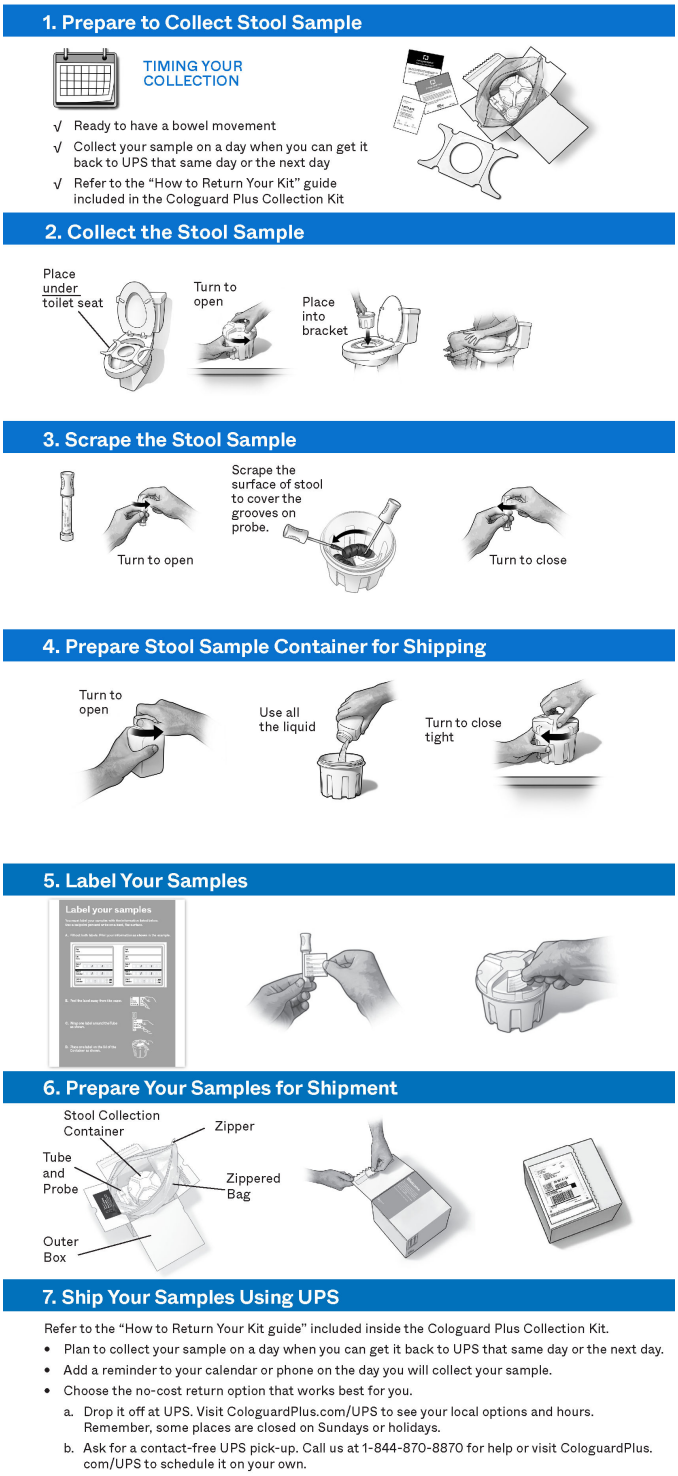


Figure 8-1: Collection Process

8.3 Interpretation of the Cologuard Plus Test Results

A negative test result means that the test did not detect signs of APL (precancer) or CRC in the stool sample. A test can also have a negative result that is

incorrect (false negative). For that reason, it is important to continue a regular screening schedule with your patients.

- Out of every 10,000 patients testing negative, approximately 2 will be falsely reassured that they do not have CRC. Out of every 100 patients testing negative, approximately 7 patients will be falsely reassured that they do not have APL.

A positive Cologuard Plus test result means that the test detected possible signs of precancer or CRC in the stool sample. A test can also have a positive test that is incorrect (false positive). Any positive result should be followed by a colonoscopy.

- Out of every 100 patients testing positive, approximately 3 patients will have CRC, 34 patients will have APL, 33 will have a non-advanced adenoma, and 30 will have no neoplastic findings.

In some cases, the Cologuard Plus test may not generate a result. If this occurs, a new patient sample may be requested.

9 References

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