Cologuard® Clinician Brochure

Intended Use and Indications for Use

The Cologuard® 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 test is performed on samples collected using the whole stool collection kit provided by Exact Sciences. A positive result may indicate the presence of colorectal cancer (CRC) or advanced precancerous lesions (APL) and should be followed by a colonoscopy. The Cologuard test is indicated to screen adults 45 years or older who are at average risk for CRC. The Cologuard test is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

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

Contraindications

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

- A personal history of CRC or APL.
- 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.

Warnings and Precautions

- Patients should not provide a sample for the Cologuard test if they have diarrhea or if they have blood in their urine or stool (e.g., from bleeding hemorrhoids, bleeding cuts or wounds on their hands, rectal bleeding, or menstruation). Unexpected bleeding should be discussed with your healthcare provider.
- Reference national guidelines for the recommended screening ages for CRC.[5] The decision to screen persons over the age of 75 should be made on an individualized basis in consultation with your healthcare provider. The Cologuard test results should be interpreted with caution in older patients as the rate of false positive results increases with age.
- The Cologuard test may produce false negative or false positive results. A false positive result occurs when the Cologuard test produces a positive result, even though a colonoscopy will not find CRC or APL. A false negative result occurs when the Cologuard test does not detect an APL or CRC even when a colonoscopy identifies either of these findings.
- A negative Cologuard test result does not guarantee absence of CRC or APL. Patients with a negative Cologuard test result should be advised to continue participating in a colorectal cancer

screening program at the appropriate guideline recommended intervals.

- The initial performance of the Cologuard test has been established in a cross-sectional study (i.e., single point in time). Programmatic performance of the Cologuard test (i.e., benefits and risks with repeated testing over an established period of time) was assessed in a longitudinal study over a three-year period.[18] Non-inferiority or superiority of the Cologuard test's programmatic sensitivity as compared to other recommended screening methods for CRC and APL has not been established.
- The clinical validation study was conducted in patients 50 years of age and older. The Cologuard test performance in patients ages 45 to 49 years was estimated by sub-group analysis of near-age groups.
- To ensure integrity of the sample, the laboratory must receive the patient samples within 144 hours of collection. **Patients should collect their sample when they can get it back to UPS on the same day or the next day.** Patients should refer to the instructions provided in the collection kit or ask their prescriber for more information.
- Patients should be advised of the caution listed in the collection kit instructions. Patients should NOT drink the preservative liquid.
- The risks related to using the 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.

RX Only

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The Cologuard Test Overview

The Cologuard test uses advanced multiple-marker, stool DNA technology to detect colorectal cancer (CRC) and advanced precancerous lesions (APL) hereafter referred to as advanced adenomas (AA). The Cologuard test is 92% sensitive for detection of CRC. The Cologuard test is a statistically superior noninvasive stool test for detecting CRC and AA, as shown in a head-to-head, cross-sectional clinical study of the Cologuard test and a commercially available fecal immunochemical test (OC FIT-CHEK, Polymedco, Inc.) ("FIT"). In the study, the Cologuard test specificity was 87% (the specificity calculation excluded both CRC and AA), which is lower than that of FIT.

The Cologuard test is designed to analyze patients' stool for the presence of 11 molecular markers, including hemoglobin and DNA markers, which may indicate the presence of colorectal cancer or advanced adenomas. Because cellular exfoliation of DNA into stool occurs continuously, the Cologuard test can detect pre-malignant neoplasia at early onset of abnormality.

Based on combined results of all of the DNA markers and hemoglobin, a single Cologuard result is determined. Cologuard test results are qualitative, positive or negative. A patient with a positive result should be referred to a colonoscopy. A patient with a negative result should continue with a regular screening schedule. If no result is obtained, a second stool collection may be requested.

Patient Samples for the Cologuard Test

Patients are not required to undergo bowel preparation or follow dietary or medication restrictions in order to complete the test. Patients follow the instructions received with the collection kit, consisting of a container for collection of stool for DNA testing and a separate sampler for collection of stool for hemoglobin testing. Both of these stool samples are required to obtain a Cologuard result. Samples are sent to a qualified laboratory for processing and testing.

The Cologuard Test Patient Navigation Program

The Cologuard 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 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 collection kit. This program also provides tracking for healthcare providers so they can measure and monitor patient adherence to the Cologuard test screening.

The Colorectal Cancer Overview

Colorectal cancer (CRC) is the second leading cause of death from cancers affecting both men and women in the United States. One in 24 Americans will suffer from CRC during their lifetime.[1] 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.[4-6] The 2021 US Preventive Services Task Force recommendation concludes that initiating CRC screening at age 45 provides moderate certainty of moderate net benefit,[5] whereas the 2018 guideline update from the American Cancer Society gave a qualified recommendation to initiate screening at age 45 in all individuals.[4] In addition, the American College of Gastroenterology (ACG) updated its CRC screening guidelines in 2021 to recommend initiation of screening at age 45 for average risk individuals.[6] The ACG guideline is a conditional recommendation based on very low-quality evidence.[6]

Approximately 40% of adults 45 years and older are not current with recommended CRC screening.[1] Half of

adults 50-54 years of age and only 20% of adults ages 45-49 report recent screening for CRC.[1]

Detection of potentially pre-malignant lesions, also known as advanced adenomas (AA), is essential for CRC prevention. Advanced adenomas 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]

Device Description

The Cologuard test utilizes a multi-target approach to detect DNA and hemoglobin markers associated with CRC, as well as pre-malignant colorectal neoplasia (i.e., AA). Three independent categories of biomarkers are targeted and provide an additive association with CRC and pre-malignant colorectal neoplasia

The first category of biomarkers involves epigenetic DNA changes characterized by aberrant gene promoter region methylation. The specific methylated gene targets include N-Myc Downstream-Regulated Gene 4 (*NDRG4*) and the Bone Morphogenetic Protein 3 (*BMP3*).[10,11] *NDRG4* and *BMP3* have been shown to be hypermethylated in CRC.[10,11] The Cologuard test procedure incorporates bisulfite conversion of non-methylated cytosine residues to uracil in the DNA sequence to enable sensitive detection of hypermethylated *NDRG4* and *BMP3.*

The second category targets specific DNA point mutations in the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene, which encodes a small GTPase that is activated transiently as a response to extracellular stimuli or signals.[12-14] *KRAS* mutations have been detected in up to 35% of CRC[15] and the 7 mutations in Exon 2 detected by the Cologuard test account for 98% of *KRAS* mutations.[15,16] *KRAS* mutations, along with *NDRG4* and *BMP3* methylation markers, have been shown to be detected in the stool of subjects with colorectal neoplasia, including subjects with CRC and pre-malignant lesions. [17]

The third category of biomarker is non-DNA based and detects hemoglobin, which can be associated with colonic bleeding. Results from the methylation, mutation, and hemoglobin assays are combined in the laboratory analysis to determine a positive or negative reportable result or no result.

Assay Technology

The patient stool samples are processed at the laboratory to isolate the DNA for testing. Amplification and detection of methylated target DNA (*NDRG4, BMP3*), *KRAS* point mutations, and *ACTB* (a reference gene for quantitative estimation of the total amount of human DNA in each sample) is performed using the Quantitative Allele-specific Real-time Target and Signal Amplification (QuARTS™) technology. Multiplexed QuARTS reactions are processed using a real-time cycler with each marker (*NDRG4, BMP3, KRAS,* and *ACTB*) monitored separately through independent fluorescent detection channels. 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 QuARTS assays and hemoglobin assay are tested along with patient samples to show that the process has been performed appropriately. Results from the methylation, mutation, and hemoglobin assays are combined during analysis to determine a positive result, negative result, or no result.

Clinical Study: Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer (DeeP-C)

Overview

The Cologuard test was the subject of a prospective, multi-centered, pivotal trial, Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer: DeeP-C Study, ("DeeP-C" or "the study"). A total of 12,776 patients were enrolled from 90 sites, including both colonoscopy centers and primary care sites. The results of the study demonstrated the safety and effectiveness of the Cologuard test as a screening test for the detection of markers associated with the presence of CRC and colorectal neoplasia. The Cologuard test demonstrated 92.3% CRC sensitivity and 86.6% specificity (specificity in this study excludes CRC and AA), 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 results exceeded the prospectively specified sensitivity threshold by nearly 20%. The study further compared CRC and AA detection by the Cologuard test to a commercially available fecal immunochemical test (OC FIT-CHEK, Polymedco, Inc.) ("FIT"), successfully demonstrating superiority for CRC ($p=0.0018$) and AA ($p<0.001$) sensitivity.

Study Design

The study was designed to enroll subjects of either sex between the ages of 50 and 84 years (inclusive), who were at average risk for development of CRC and asymptomatic for gastrointestinal symptoms warranting diagnostic colonoscopy. In addition, subject enrollment was age-weighted toward a slightly older population to increase the point prevalence of CRC in this study. Sixty-four percent of subjects in the actual study population were of age 65-84.

Subjects participating in the pivotal trial provided a stool sample and subsequently underwent colonoscopy within 90 days of study enrollment. Subjects collected stool samples for Cologuard and FIT testing at home. Subjects then underwent colonoscopy per standard of care. Subjects and physicians remained blinded to the results of the Cologuard and the FIT tests. Results from the Cologuard and the FIT tests were compared to the results of the colonoscopy examination and histopathologic diagnosis of all significant lesions either biopsied or removed.

Negative colonoscopy findings were categorized as negative (Table 1, category 6.2). 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 1. Sensitivity analysis was performed using positive findings in categories 1 and 2 while specificity was calculated using categories 3 through 6 (all findings excluding CRC and AA).

Table 1: Category definitions

Category	Findings
1	CRC, all stages (I-IV)
2	Advance adenoma, including the following subcategories: 2.1 – Adenoma with carcinoma in situ/high grade dysplasia, any size 2.2 – Adenoma, villous growth pattern (≥25%), any size 2.3 – Adenoma ≥ 1.0 cm in size, or 2.4 – Serrated lesion, ≥ 1.0 cm in size
3	1 or 2 adenoma (s), >5 mm in size, or < 10 mm size, non-advanced
4	≥ 3 adenomas, <10 mm, non-advanced
5	1 or 2 adenoma(s), ≤5 mm in size, non-advanced
6	Negative – No neoplastic findings 6.1 – negative upon histopathological review 6.2 – no findings on colonoscopy, no histopathological review

Study Population and Baseline Demographics

Study enrollment and population demographics are summarized in Figure 1. A total of 10,023 subjects with colonoscopy and the Cologuard test data were included in the primary analysis population. This population included 65subjects with CRC. Analysis was conducted to rule out bias associated with the subjects excluded from the analysis population.

The average age of subjects included in the primary analysis was 64.2 years, and there were a slightly higher percentage of female subjects (5,378/10,023, 53.7%) as compared with male subjects (4,645 /10,023, 46.3%). Two 49-year-old subjects and one 44-year old subject were included in the study, which was inconsistent with theintended user population. Each of these subjects was a true negative on the Cologuard test and their inclusion did not notably impact data analyses. The majority of subjects were White (8,422/10,023, 84.1%), although 10.7% of the population were Black or African American subjects (1,071/10,023). Nearly 10% of subjects were Hispanic or Latino (991/10,023, 9.9%). Average BMI was 28.8 and the majority of subjects never smoked (5.531/10,023, 55.2%).

Figure 1: Clinical Study Demographics

Total Enrollment	**12,776**
Primary Endpoint Valid Cologuard test + Colonoscopy	**10,023**
Secondary Endpoint Valid Cologuard test + FIT + Colonoscopy	**9,989**

Demographics		
Age (Years)	Average	64.2
	Range	44-84
Gender	Male	46.3%
	Female	53.7%
BMI	Average	28.8
	Range	13.3-68.2
Ethnicity	Hispanic/Latino	9.9%
	Non Hispanic/Latino	90.1%
Race	White	84.1%
	Black	10.7%
	Asian	2.6%
	Amer. Ind/Alaskan Native	0.4%
	Native Hawaiian/Pacific Islands	0.2%
	Other	2.1%
Smoking History	Never Smoked	55.2%
	Former Smoker	35.8%
	Smoker	9.0%

Clinical Performance Measures

The primary and secondary performance measures for the clinical study are summarized in Table 2 below. The primary performance measures were the sensitivity and specificity of the Cologuard test for CRC, using colonoscopy with histopathology as the reference method. The primary analysis required that the lower bound of the 95% one-sided confidence interval for the sensitivity of the Cologuard test for CRC exceed 65%. The specificity analysis for CRC required that the lower bound of the one-sided 95% confidence interval exceed 85%.

With respect to the secondary performance measure, the Cologuard test was compared to FIT using a non-inferiority test for CRC sensitivity and using a superiority test for advanced adenoma (AA) sensitivity. In order for the Cologuard test to be deemed non-inferior to FIT, the one-sided 95% confidence interval lower bound for the Cologuard test – FIT difference in percentages with a positive test among subjects with CRC was required to exceed -0.05. Establishing superiority required a one-sided p-value <0.025 (exact McNemar's comparison test).

Table 2: Clinical Study Primary and Secondary Performance Measures

Primary Performance measures	• Determine the CRC sensitivity and specificity of the Cologuard test.
Secondary Performance measures	• Compare the Cologuard test to FIT for CRC and AA sensitivity.

Summary of Clinical Study Results

Results from the clinical study demonstrated that the Cologuard test successfully met the primary performance measure of the study, establishing a clinically meaningful sensitivity and specificity for CRC. Sensitivity of the Cologuard test for CRC was 92.3% (60/65) with a one-sided 95% confidence interval lower bound of 84.5, substantially exceeding the protocol-specified threshold of 65%. In addition, the Cologuard test successfully demonstrated a clinically meaningful specificity according to the protocol-specified criteria. The specificity of the Cologuard test was 86.6%, with a one-sided 95% confidence interval lower bound of >86.0%.

Clinical study results demonstrated superiority of the Cologuard test to FIT for sensitivity in detecting CRC. Secondary performance measures included an analysis of performance of the Cologuard test and FIT using colonoscopy as a reference. The Cologuard test correctly detected 60 of the 65 total CRC cases identified by colonoscopy (92.3%). FIT captured only 48 of the 65 CRC cases identified by colonoscopy (73.8%). FIT identified only a single cancer that was not identified by the Cologuard test. The Cologuard test, meanwhile, identified 13 cancers that were missed by FIT. The Cologuard test was compared to FIT using a non-inferiority test for CRC sensitivity. In addition, the Cologuard test demonstrated superiority over FIT with respect to sensitivity for CRC using an exact McNemar's comparison test as the one-sided p-value (p=0.0018) was well below the p <0.025 threshold for superiority. The lower bound of the one-sided confidence interval for the Cologuard – FIT difference was 0.080, substantially exceeding the protocol-specified non-inferiority threshold of -0.05.

Establishing superiority for AA sensitivity required a one-sided p-value <0.025 (exact McNemar's comparison test). The Cologuard test demonstrated superiority for AA sensitivity, with a p-value of <0.001, substantially below the threshold for superiority of p<0.025. FIT identified only 29 AA cases that were not captured by the Cologuard test, while the Cologuard test identified 170 AA cases that were not positive on the FIT test.

Analysis was also performed to calculate the Cologuard test's negative predictive value (NPV) for Category 1 (CRC) and Category 2 (AA). Clinical results show that a negative patient result for the Cologuard test gives 99.94% assurance that the patient does not have cancer and a 94.79% chance that the patient does not have an advanced adenoma.

The Cologuard Test and FIT Performance Comparison

The Cologuard test was superior to FIT for both CRC and AA detection. The Cologuard test also demonstrated high sensitivity fordetection 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 test demonstrated a numerically greater sensitivity than FIT for detection of CRC and AA across lesion subgroups. Sensitivity results are summarized in Table 3 and Table 4 below. As noted above, the Cologuard test specificity was 86.6% and FIT specificity was 95%. These specificity measures excluded CRC and AA for both tests.

The Cologuard test sensitivity for stage I cancer was 89.7% compared to 65.5% for FIT (p=0.039). Sensitivity for stage II cancer was 100.0% for the Cologuard test compared to 76.2% for FIT (p=0.062). CRC sensitivity was also compared to FIT by size of the lesion, with higher detection at each lesion size than FIT. When analyzed by lesion location, the Cologuard test showed 90.0% sensitivity for proximal cancer compared to 66.7% for FIT (p=0.039). The Cologuard test also detected higher risk precancerous lesions, including high grade dysplasia (69.2% Cologuard, 46.2% FIT, p=0.004) and sessile serrated polyps (43.0% Cologuard, 5.1% FIT, p<0.001). The Cologuard test and FIT were both better at detecting precancerous lesions as lesion size increased from 0.5 cm to ≥3 cm (value for trend for bothwas p<0.001).

Table 3: Cologuard and FIT Cancer Sensitivity

Subgroup	n=	Cologuard Sensitivity	FIT Sensitivity
Cancer Stage			
CRC, all stages (p=0.002)	65	92.3%	73.8%
Stage I (p=0.039)	29	89.7%	65.5%
Stage II (p=0.062)	21	100.0%	76.2%
Stage III	10	90.0%	90.0%
Stage IV	4	75.0%	75.0%
Stage I-III (p=0.002)	60	93.3%	73.3%
Cancer Size			
< 5 mm	0	0	0
5-9 mm	5	80.0%	60.0%
10-19 mm	14	92.9%	85.7%
20-29 mm	12	91.7%	66.7%
≥30 mm	34	94.1%	73.5%
Cancer location			
Proximal (p=0.039)	30	90.0%	66.7%
Distal (p=0.062)	35	94.3%	80.0%

*Cologuard specificity was 86.6% and FIT specificity was 95%. These specificity measures excluded CRC and AA for both tests.

Table 4: Cologuard and FIT Advanced Adenoma Sensitivity

Subgroup	Cologuard n=	Cologuard Sensitivity	FIT n=	FIT Sensitivity
Pre-malignant Neoplasia				
AA, all subcategories (p<0.001)	760	42.4%	757	23.8%
High grade dysplasia (p=0.004)	39	69.2%	39	46.2%
Sessile serrated ≥10 mm (p<0.001)	100	43.0%	99	5.1%
AA location				
Proximal (p<0.001)	433	33.0%	431	15.5%
Distal (p<0.001)	326	54.6%	325	34.8%
Lesion Size	p value for trend<0.001		p value for trend<0.001	
< 5 mm	10	20.0%	10	20.0%
5-9 mm	56	32.1%	56	14.3%
10-19 mm	577	39.0%	574	20.9%
20-29 mm	79	64.6%	79	43.0%
≥30 mm	38	68.4%	38	42.1%

*Cologuard specificity was 86.6% and FIT specificity was 95%. These specificity measures excluded CRC and AA for both tests.

The Cologuard Test Subgroup Analysis: please note that the clinical study was not designed to evaluate subgroups and subgroup analysis should be interpreted with that in mind.

The clinical study results were analyzed according to various demographic characteristics, including gender, age, and race/ethnicity as summarized in Table 5 below. Although CRC sensitivity was higher for males versus females and higher in Whites and Asians compared to Black/African Americans, AA sensitivity and specificity remained consistent across subgroups, with only a few differences likely attributed to a lower number of subjects from all subpopulations in the study.

The Cologuard test CRC sensitivity was higher for males versus females. Meanwhile, specificity of the Cologuard test was similar for females as compared with males. Specificity was 87.3% (4,398/5,037) for females, compared with 85.8% (3,569/4,161) for male subjects. Advanced adenoma detection showed similar results between males and females.

For age, the Cologuard test sensitivity for CRC was consistently high across all age groups. Sensitivity for patients 65 years of age and older ranged from 88.9% to 100.0%. Although sensitivity was 75% for subjects age 60-64, thenumber of CRC cases was particularly small in this age group (n=4); only one CRC case was not detected by the Cologuard test. With respect to AA, sensitivity was similar across all age groups, with sensitivity as high as 46.8% for subjects between the ages of 70 and 79. The Cologuard test specificity for CRC was also high across all age groups.Specificity was in the 80% range or above for most age groups, aside from subjects >75 years old. Specificity for AA was also similar across age groups.

The Cologuard test CRC sensitivity was very high among White subjects, but lower among Black or African American subjects) and high among the small number of Asian CRC cases. However, the results observed in Black/AfricanAmerican subjects may have been affected by the low overall number of cancer cases in that subpopulation. Sensitivity among Hispanic or Latino subjects was high, although the sample size was small.

The Cologuard test sensitivity for AA was similar for White and Black/African American subjects. Sensitivity was also similar among Hispanic/Latino subjects. AA sensitivity was lower among Asian subjects and very high for American Indian or Alaskan Natives, compared with other groups. Only the American Indian and Alaskan Native subpopulations showed higher sensitivity in AA detection. Differences between racial and ethnic subpopulation results may be affected by the small number of subjects in the African American and American Indian or Alaska Native subpopulations. Specificity for the Cologuard test was high across all racial and ethnic groups, with rates >85% for most groups.

Table 5: Cologuard test Performance by Subgroup

Subgroup	CRC Sensitivity	AA sensitivity	Specificity
Gender			
Male	34/34 (100%)	201/450 (44.7%)	3569/4161 (85.8%)
Female	26/31 (83.9%)	121/310 (39%)	4398/5037 (87.3%)
Age			
<60 yrs	7/7 (100.0%)	65/171 (38.0%)	2491/2703 (92.2%)
60-64 yrs	3/4 (75.0%)	24/57 (42.1%)	681/765 (89.0%)
65-69 yrs	19/20 (95.0%)	125/301 (41.5%)	2871/3352 (85.7%)
70-74 yrs	16/18 (88.9%)	72/154 (46.8%)	1292/1566 (82.5%)
75-79 yrs	6/6 (100.0%)	29/62 (46.8%)	480/617 (77.8%)
>79 yrs	9/10 (90.0%)	7/15 (46.7%)	152/195 (77.9%)
Race			
White	53/55 (96.4%)	271/641 (42.3%)	6639/7726 (85.9%)
Black or African American	5/8 (62.5%)	36/85 (42.4%)	879/978 (89.9%)
Asian	1/1 (100.0%)	4/13 (30.8%)	229/245 (93.5%)
American Indian or Alaska Native	0/0	3/4 (75.0%)	24/32 (75.0%)
Native Hawaiian or Other Pacific Islander	0/0	0/0	21/23 (91.3%)
Other	1/1 (100.0%)	7/16 (43.8%)	171/189 (90.5%)
Ethnicity			
Hispanic or Latino	8/9 (88.9%)	23/59 (39.0%)	837/923 (90.7%)
Not Hispanic or Latino	52/56 (92.9%)	298/700 (42.6%)	7127/8272 (86.2%)

Performance in Age Group 45 to 49

The US Preventive Services Task Force on Screening for Colorectal Cancer (2021) and the American Cancer Society Colorectal Cancer Screening Guideline (2018) lowered the recommended age to start colorectal cancer screening from 50 to 45 for patients at average risk for CRC and included the use of the multi-target stool DNA test (the Cologuard test) for cancer screening within that recommendation, along with other stool-based non-invasive tests and structural (visual) examination options, depending on patient preference and test availability.[4,5] Both organizations based their recommendations on colorectal cancer (CRC) incidence and mortality rates, results from microsimulation modeling that demonstrate a favorable benefit-to-burden balance of screening beginning at age 45, and the expectation that screening will perform similarly in adults ages 45 to 49 as it does in adults ages 50 and older.[4,5]

Retrospective analysis of Cologuard test results in patients 45 to 49

Retrospective data were collected to evaluate whether the Cologuard test performance in samples from patients ages 45 to 49 years is comparable to that achieved in samples obtained from patients ages 50 and older. Through September 2018, there had been 2241 completed Cologuard tests (through Exact Sciences Laboratories) aged45 to 49 years. It is unknown if these patients were at average risk. Of these tests, 7.4% (165/2241) had a positive result and 92.6% (2076/2241) had a negative result, indicating the specificity in this age group is ≥92.6%, which is comparable to the specificity of patients ages 50 to 59 from the DeeP-C study. Follow-up data were notavailable from the 2241 completed Cologuard tests to confirm colorectal cancer outcomes for either positive or negative results.

Post- Approval Study

Summary of the Post-Approval Study Methods

Study Objective

The Cologuard test was the subject of a prospective, longitudinal, multi-center post-approval study (PAS),[18] as required by the FDA in approval order dated August 11, 2014 for PMA130017. A total of 2,321 patients were enrolled from 40 sites within the US including both private-practice and academic settings. The study was designed to collect longitudinal data to assess the performance of Cologuard testing when repeated 3 years after the prior test in patients at average risk of CRC.

Study Design

This was a prospective, longitudinal study designed to assess the clinical impact of repeat testing with the Cologuard test at a 3-year interval in average risk patients. Enrolled participants were prescribed the Cologuard test per approved labeling at baseline (T0). Participants with a positive Cologuard result at T0 were referred to colonoscopy and study participation was completed. Participants with a negative Cologuard result at T0 remained in the study, and were evaluated for changes in medical history at year 1 (T1) and year 2 (T2). Participants who met the inclusion and exclusion criteria of the study at year 3 (T3) repeated the Cologuard test and underwent a colonoscopy, regardless of the Cologuard test outcome. Participation of patients who had a colonoscopy at any time during the study was discontinued following collection of the colonoscopy data and associated histopathology results.

Key study Endpoints

The study was designed to evaluate whether repeat testing 3 years after initial testing would provide additional information beyond that provided by baseline Cologuard testing. That study objective was evaluated with a primary endpoint that sought to confirm that the positive predictive value (PPV) at three years (T3) is greater than 1 minus the negative predictive value (NPV) at T3. This endpoint is intended to demonstrate that Cologuard testing at T3 detects CRC/ AA arising within 3 years following a negative test result at baseline (T0). The primary effectiveness endpoint was to be calculated for CRC alone and for the combined CRC and AA (CRC+AA) group. The secondary endpoint was the observed vs. the expected reduction in CRC incidence at T3.

The primary endpoint analysis was not powered to demonstrate the value of repeat testing for CRC.

Study Population and Detection

Enrollment was completed on July 27, 2016, with a final enrollment count of 2,321 participants, consistent with the planned sample size of the study. The studied population consisted of men and women between the ages of 50 and 84, inclusive, at average risk of developing CRC. Study enrollment was age-weighted to enhance enrollment for participants 65 years of age and older, with this age group ultimately representing 67% of the total enrolled population. During initial planning of the study, both Exact Sciences and FDA recognized that follow-up compliance might be challenging. Given this expected issue, the total study size population accounted for the possibility of 15%

drop out per year and 15% refusal of colonoscopy at T3. Significant efforts were made during the course of the study to retain subjects and encourage compliance with the study procedures.

Of the 2,321 participants enrolled in the study at 40 sites, 1,760 participants were eligible for the study based on a negative Cologuard test result at T0. Of the 1,760 participants with a negative Cologuard test result at T0, 1,645 eligible participants (93.5%) completed the T1 office/phone visit, 1,510 (87.1%) participants completed the T2 office/phone visit, and 1,223 (75.7%) provided some data at the T3 timepoint. Of these patients, 1,067 (60.6%) adhered to repeat Cologuard testing at T3 and 591 obtained colonoscopies. Study attrition was 66% ((1760-591)/1760). The Cologuard test accurately detected 22 of the 63 advanced adenomas (Category 2). There were no CRCs observed in the study set. The overall results of the study are shown in the Table 6 below:

Table 6: Post Approval Study Results

	Colonoscopy/Biopsy		
	Advanced Adenomas*	Non-Advanced or no finding	
Cologuard result at T3			Total
Positive	22	100	122
Negative	41	428	469
Total	63	528	591

*No CRCs were observed in the study set at T3

Summary of the Post-Approval Study Results

Final Safety findings (key endpoints)

Safety monitoring analyses were conducted once all participants who remained after T0 had reached the T1 milestone (Year 1 Safety Analysis) and through the T2 milestone (Year 2 Safety Analysis). The purpose of the analyses was to assess whether the rate of newly diagnosed CRCs between T0 and T3 was unexpected and warranted study termination. In those analyses, the study was to be terminated if the cumulative number of new CRCs was greater than the one-sided 95% upper bound specified in the protocol (6 CRCs at Year 1). Only 1 CRC was identified during the study period, which met the criteria for study continuation.

Of the 42 deaths reported for study participants, none are known to be related to either the Cologuard test or CRC. Additionally, no adverse events were reported. Given that no CRCs were detected at T3, only the CRC+AA analysis was conducted and was limited to AAs. The effectiveness endpoint was met, with the difference between the T3 PPV and 1 minus the T3 NPV of 9.29% (95% CI 1.83%, 17.63%) This result statistically ruled out a difference of zero (p=0.0124), meeting the endpoint success criterion. This represents an approximately 9% net gain in certainty of disease status after performing the repeat Cologuard test at T3, beyond what could be determined by disease prevalence alone.

The primary endpoint analysis was not powered to demonstrate the value of repeat testing for CRC; however, it demonstrated with statistical significance that the test PPV at T3 was greater than 1 minus the T3 NPV for composite outcome of CRC or AA. The Cologuard test is informative when used for repeat testing for composite outcome of CRC or AA three years after initial testing.

The study secondary endpoint evaluated the observed versus expected reduction in CRC incidence at T3. A total of 5 CRC events were expected at T3 with an assumed CRC incidence rate of 0.00686. However, only 1 case of CRC occurred after T0, giving an observed incidence after a negative T0 Cologuard of 0.001374, numerically less than expected (two-sided p=0.0729 or one-sided p=0.0402).

Thus, the study met its predefined primary endpoint for the composite outcome of CRC or AA but was not powered to be conclusive for CRC alone and demonstrated that the test is informative for the composite outcome of CRC or AA when repeated after a three-year period.

Study Strength and Limitations

The primary strength of the study is that it is the first of its kind and provides novel data regarding repeat performance of the Cologuard test.

This study met its predefined primary endpoint for the composite outcome of CRC or AA as it was not designed to be conclusive for CRC alone. This study demonstrated that the test is informative for the composite outcome of CRC or AA when repeated after a three-year period.

The first limitation of the study was that the study goal was to demonstrate that the test met a pre-specified Predictive Summary Index (PSI) at T3, rather than to establish a particular screening interval. The study was prospectively designed with pre-specified expectations regarding PPV and NPV at the final testing timepoint and did not as a priority incorporate re-testing considerations.

Another limitation of this study was that follow up was challenging and a significant percentage of participants did not reach each designated study phase, 33.5% patients (591 out of 1,760) had both valid Cologuard test and colonoscopy at year 3 for primary study endpoint analyses. No statistical adjustments were conducted for multiplicity. Several measures were implemented to increase compliance. Although final sample size did not align with the predicted size, a sufficient number of participants completed colonoscopy to allow for primary endpoint analysis, resulting in meaningful data about the safety and effectiveness of Cologuard. To support the conclusion that the missing data did not introduce additional bias in the study, four different data analyses were conducted and concluded that the performance observed accurately reflects the clinical performance of the Cologuard test at a 3-year timepoint:

1. Having quantified that participants with a positive T3 Cologuard were more motivated to undergo a colonoscopy.
2. Confirmed race, age, and T3 Cologuard result as factors influencing the decision to undergo screening colonoscopy.
3. Using the method of Kosinski et al.[19] to estimate all possible sensitivity and specificity values across a range of assumed prevalence of disease in unverified individuals to account for Missing at Random (MAR).
4. Using age, race, and T3 Cologuard findings, we imputed consistent mean values for sensitivity, specificity, PPV, and NPV using SAS PROC MIANALYZE to control for age, race, and T3 Cologuard result; the retrospective power for this analysis was 85% using all 1,036 participants.

The final limitation was that participants with positive Cologuard results at T0 were referred to colonoscopy and their study participation was completed at that time. If these participants were followed through T3, these data would have provided additional information about the test.

Ordering the Cologuard Test

The Cologuard test is available for physicians to order through 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 www.cologuardhcp.com. The Cologuard test includes a patient navigation program that provides attentive service to physicians and patients with live specialists. For any questions about the Cologuard 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
844-870-8870

Sample Collection

- Samples for use with the Cologuard test must be collected with the collection kit including a stool sample for DNA testing (Container) and a stool sample for Hemoglobin testing (Tube).
- 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 menstruation.
- Patients should familiarize themselves with information provided with the collection kit 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.
- Patients should collect their sample when they can get it back to UPS on the same day or the next day. Patients should send stool samples to the laboratory according to the instructions provided with the collection Kit.

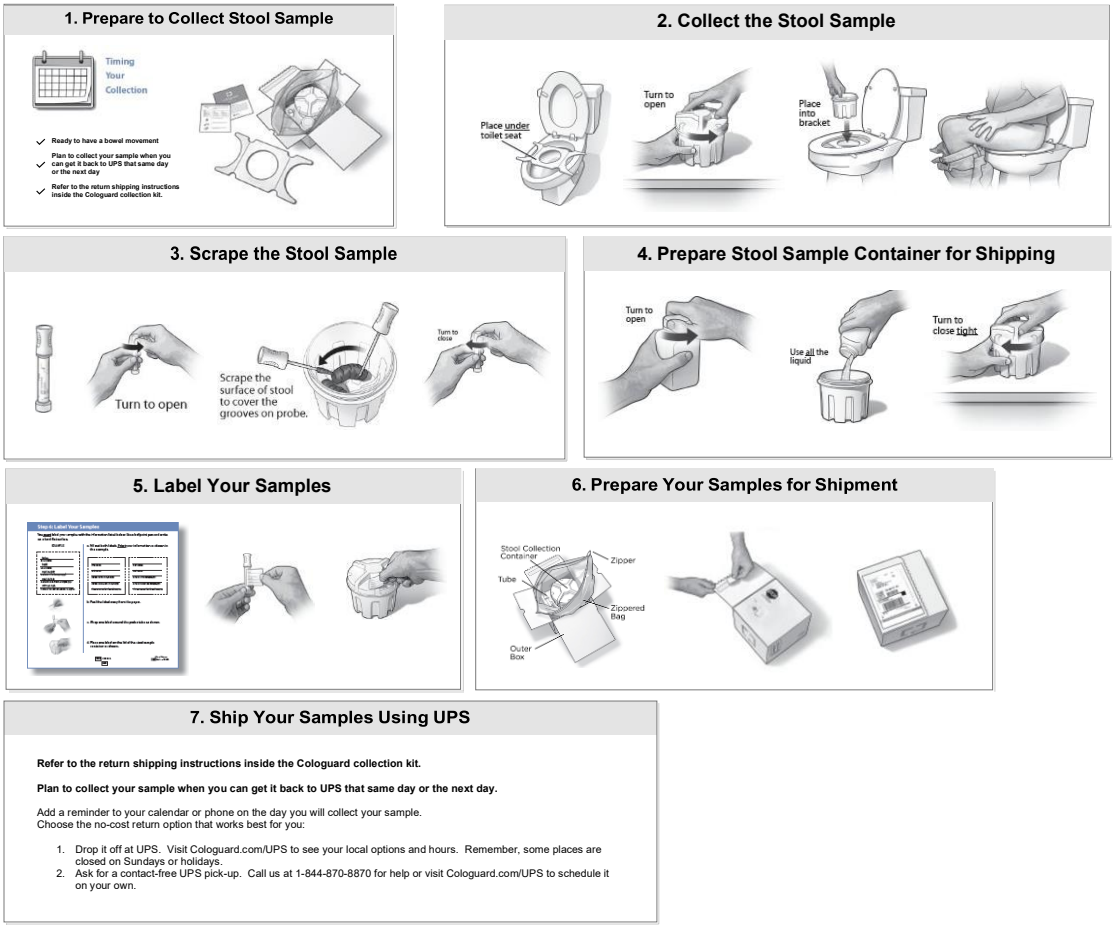
Interfering Substances

There are no known interfering substances with the Cologuard 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, anti-acids, upset stomach relief products, urine, alcohol, common vegetables and fruits, fats, and lipids.There was no observed interference with any substance in either assay. The hemoglobin assay was also testedwith antibiotics, anti-inflammatories, anti-fungal drugs, pain relievers, and decongestants with no observed interference. The molecular assay was additionally tested with animal genomic DNA of commonly edible animals(both high and low levels) with no observed interference.

Instructions for Sample Collection

Once the Cologuard test has been ordered, the patient will receive a collection kit. Instructions for patient specimen collection are provided in the 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 instructions to ensure that any patient questionsare addressed. An overview of the collection process is provided in the figure below.

Figure 5: Patient sample collection process



Interpretation of the Cologuard Test Results

A negative test result means that the test did not detect signs of 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.

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

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