

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Multi-Target Stool DNA (mt-sDNA)-Based Colorectal Cancer Screening Test
Device Trade Name:	Cologuard Plus™
Device Procode:	PHP
Applicant's Name and Address:	Exact Sciences Corporation 5505 Endeavor Lane Madison, WI 53719
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P230043
Date of FDA Notice of Approval:	October 3, 2024

II. 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.

III. CONTRAINDICATIONS

Cologuard Plus is NOT indicated for use in patients who have the following:

- A personal history of colorectal cancer or advanced precancerous lesions.
- A positive result from another colorectal cancer 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 colorectal cancer:
 - 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.

IV. 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. 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 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 negative Cologuard Plus test result does not guarantee the absence of cancer or advanced precancerous lesions. 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.

- Read and understand the Safety Data Sheets (SDSs) for the reagents before storing, handling, or working with any chemical or hazardous material. SDSs are available by contacting Technical Services (refer to Contact Information or contact the original reagent manufacturer for other materials for guidance on storage, safe handling, disposal). Fecal samples should be treated as if they are potentially infectious.

V. **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.^{1,2,3} 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 Exact Sciences Analysis Software to determine a Positive or Negative result or an Invalid result.

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.

The Cologuard Plus Collection Kit

Cologuard patient guide

Container for collection of stool for DNA testing

Sampler (Tube) for collection of stool for hemoglobin testing

The Cologuard Plus Test Reagents

The reagents used by the laboratory in the Cologuard Plus test workflow are listed below, grouped together based on the element of the workflow they support. Additional information may be found in the Cologuard Plus Instructions for Use.

- DNA capture reagents
 - CG2 Capture Beads (CG2 CAP BDS)
- DNA preparation reagents
 - CG2 Denaturation Solution (CG2 DEN SLN)
 - CG2 Bisulfite Conversion Solution (CG2 BIS SLN)
 - CG2 Binding Beads (CG2 BND BDS)
 - CG2 Desulphonation Solution (CG2 DES SLN)
 - CG2 Carrier Solution (CG2 CAR SLN)
- Molecular Assay reagents
 - CG2 Elution Buffer (CG2 ELU BFR)
 - CG2 Oligo Mix (CG2 MIX)
 - CG2 Enzyme Mix (CG2 ENZ)
 - CG2 DNA Calibrator 1, High (CG2 D CAL 1)
 - CG2 DNA Calibrator 2, Mid (CG2 D CAL 2)
 - CG2 DNA Calibrator 3, Low (CG2 D CAL 3)
- Hemoglobin Assay reagents
 - CG2 Hb Capture Beads (CG2 BEAD)
 - CG2 Antibody Conjugate (CG2 CONJ)
 - CG2 Substrate (CG2 SUBS)
 - CG2 Stop Solution (CG2 STP SLN)
 - CG2 Hemoglobin Assay Calibrator (CG2 CAL)
- Ancillary materials
 - Inhibitor Removal Tablet (TABLT)
 - Spin Filter (FILT)
 - Capture Solution (CAP SLN)
 - CG2 Capture Wash (CG2 CAP WSH)
 - CG2 Binding Solution (CG2 BND SLN)
 - CG2 Conversion Wash Concentrate (CG2 CNV WSH)
 - CG2 Hb Bead based Assay Wash (CG2 WSH)
 - CG2 Reconstitution Buffer (CG2 REC BFR)
 - Stool Buffer (STL BFR)

Additionally, two sets of controls are required to be run alongside patient samples in the Cologuard Plus test workflow to ensure proper functioning of the test—one for the Molecular Assay and one for the Hemoglobin Assay. These are listed below:

- Cologuard Plus DNA controls
 - CG2 DNA Control 1, High (CG2 D CTRL 1)
 - CG2 DNA Control 2, Low (CG2 D CTRL 2)
 - CG2 DNA Control 3, Negative (CG2 D CTRL 3)
 - CG2 DNA Control 4, NTC (CG2 D CTRL 4)
- Hemoglobin Assay controls

- CG2 Hemoglobin Control 1, High (CG2 CTRL 1)
- CG2 Hemoglobin Control 2, Low (CG2 CTRL 2)
- CG2 Hemoglobin Control 3, Negative (CG2 CTRL 3)

Instruments

The instruments that are part of the Cologuard Plus System, required to perform the Cologuard Plus test and qualified by Exact Sciences under the Exact Quality System are listed in the table below (Table 1).

Table 1: Instruments Required for Cologuard Plus Assay

Instrument	Manufacturer/Supplier
Sample Mixer 2 (120V) or equivalent	Exact Sciences
Solaris™ 2000 Open Air Orbital Shaker or equivalent	Thermo Fisher Scientific or equivalent
Tube Shaker, Base 50 mL or equivalent	Exact Sciences
Tube Shaker, Rack 50 mL or equivalent	Exact Sciences
Capture Shaker Rack or equivalent	Exact Sciences
Capture Incubator 2	Exact Sciences
Capture Incubator Tube Lift	Exact Sciences
Capture Aspirator or equivalent <ul style="list-style-type: none"> • Vacuum Trap Box Kit • Vacuum Pump (optional) 	Exact Sciences
STARlet	Hamilton
HBB STARlet	Hamilton
Epoch2 Integration Kit	Hamilton
QuantStudio™ 5 Dx Real-Time PCR System	Thermo Fisher Scientific
BioTek® Epoch™ 2 Absorbance Microplate Reader, Exact Sciences Configuration	Agilent Technologies
System Computer	Exact Sciences

Additionally, the Cologuard Plus test requires an array of general laboratory equipment such as centrifuges, shakers, bottle top dispensers, and pipettes.

Software

In order to perform the automated portions of the Cologuard Plus test workflow, a combination of custom software and off-the-shelf (OTS) software is used. The custom software provides the overall test result from a proprietary algorithm that incorporates the results of DNA methylation and hemoglobin assays. It is composed of the Exact Sciences System Software v2.2 (“System Software”) and the Cologuard 2 Test Definition v1.1 (“Test Definition”).

The System Software is an assay-agnostic suite of software applications that communicates with the instruments required for Cologuard Plus test and runs the Test Definition, which dictates all assay-specific software control, data analysis, and result generation. Part of the System Software is the Home Page Software module, which Exact Sciences developed to provide an interface for the user to launch individual system software applications.

The Test Definition encompasses the assay-specific interfaces, data formats, data reduction libraries, parameters, and scripts required to direct the software through assay processing, data collection, data reduction, and interpretation of results.

The Cologuard Plus test also makes use of two instruments developed by Exact Sciences, the Capture Incubator 2 and Capture Aspirator, which also contain software and firmware.

Finally, in addition to the instrument software described above, the Cologuard Plus System utilizes several pieces of ancillary software developed by Exact Sciences. These include Password Utility, Configuration Editor, SLIB Generator, and Export Software.

Principles of the Procedure

The Cologuard Plus test is designed to analyze patients’ stool for the presence of DNA and hemoglobin markers which may indicate the presence of CRC or APL. Patients use the Cologuard Plus Collection Kit, consisting of a Container for collection of stool for DNA testing and a separate sampler (Tube) for collection of stool for hemoglobin testing. Both of these stool samples are required to obtain a Cologuard Plus result.

In the processing procedure for DNA testing, the stool sample is mixed with buffer in the Container using the Sample Mixer. An aliquot of the buffered stool sample is centrifuged to pellet solids and generate supernatant. The assay procedure begins with treatment of the supernatant with an Inhibitor Removal Tablet to remove inhibitors that may affect the detection of the DNA biomarkers. Treated supernatant is then combined with denaturing reagents and incubated with target-specific magnetic particles using the Capture Incubator instrument to capture sequences for *LASS4*, *LRRC4*, *PPP2R5C* and *ZDHHC1* (reference gene).

Using automated processes for capture aspiration and Hamilton Microlab® STARlet (STARlet) instruments, targeted sequences are separated from the solution, washed, and eluted from the particles. Eluted DNA is treated with bisulfite conversion reagents and further purified with silica-coated magnetic beads from which DNA is eluted.

The Long-probe Quantitative Amplified Signal (LQAS) technology combines real-time PCR and invasive cleavage to perform allele-specific amplification and detection of methylated target DNA in the Molecular Assay. Purified DNA is mixed with the LQAS reaction master mix and processed using a real-time cycler. Each marker is monitored separately through an independent fluorescence detection channel.

In a parallel workflow, the Hemoglobin Assay stool sample is prepared and analyzed in a quantitative Enzyme-Linked Immunosorbent Assay (ELISA) that determines the concentration of hemoglobin in the sample. Each sample is added to a single well of a 96-well deep well plate (DWP) and combined with magnetic capture beads pre-coupled with anti-hemoglobin antibody, and then washed to remove any unbound material. A second anti-hemoglobin antibody conjugated to the enzyme horseradish peroxidase (HRP) is then added to the wells and incubated with a colorimetric substrate for HRP. After the reaction is stopped and the absorbance is read on a plate reader, the level of hemoglobin present in the stool sample is calculated using a calibration curve prepared from a set of calibrators with known hemoglobin concentrations.

Result interpretation

Run control samples for both the Molecular Assay and Hemoglobin Assay are tested along with patient samples to show that the process has been performed appropriately. CG2 DNA Controls and Hb Bead Based Controls are required in each run to obtain valid assay results. Results from the molecular and hemoglobin assays are integrated by the Exact Sciences Analysis Software to determine a Positive or Negative reportable result or an Invalid result.

Individual results could be marked as invalid for multiple reasons, including:

- An error occurred during processing on the automated platform.
- Background data collected during the LQAS PCR run was above the allowable limit.
- ZDHHC1 concentration was below the limit of 2.4 log strands.
- A sample was user-invalidated within the software due to known operator manual processing error.

In the event of an invalid test, up to two re-tests may be performed.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several alternatives for screening for CRC, including both invasive and non-invasive options. Invasive options include flexible sigmoidoscopy, computed tomography colonography (CTC), and conventional colonoscopy. Non-invasive CRC screening

options include, stool-based [multi-target stool DNA-based test, guaiac fecal occult blood test (gFOBT), fecal immunochemical test (FIT), multiple-target stool RNA-based test] and blood-based plasma DNA testing.

Colonoscopy is considered to be the most accurate screening tool available, which can involve the removal of precancerous lesions to prevent cancer.

Each alternative has its own advantages and disadvantages. Patients who have a positive or abnormal test by an invasive or non-invasive screening method, except for colonoscopy, warrant further investigation through conventional colonoscopy. A patient should discuss these alternatives with your healthcare provider to select the method that best meets the patient's needs, expectations and lifestyle.

VII. MARKETING HISTORY

The Cologuard Plus test has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a description of the potential adverse effects (e.g., complications) associated with the use of the device. Due to the nature of the noninvasive stool collection process, potential adverse effects caused by or related to stool collection are unlikely, and no adverse events related to stool collection were reported in the clinical study for the Cologuard Plus test (see Section X below). The primary risk associated with the Cologuard Plus test is a false test result (i.e., a false positive or a false negative result). All positive test results should be followed by a colonoscopy. In the instance of a false negative result, there is a possibility that a case of CRC or APL could go undetected.

IX. SUMMARY OF NONCLINICAL STUDIES

Nonclinical studies were conducted by Exact Sciences to evaluate the analytical performance characteristics of Cologuard Plus. The studies are described below.

A. Algorithm Development and Clinical Cutoff Determination

A study was conducted in order to establish an algorithm and clinical decision point (cutoff value) for the Cologuard Plus test. The study included 3,011 samples: 100 CRC, 242 APL, 813 non-advanced precancerous lesions, and 1,856 negatives. Models were fit via nominal logistic regression, general additive model, neural net, and random forest. The logistic regression model was selected as it provided the best clinical performance with the least complexity. The cross-validated results for this final algorithm were 91.9% CRC sensitivity, 40.7% APL sensitivity, 90.9% specificity for negatives alone, and 88.5% for negatives and non-APL; these values aligned with point estimates.

B. Analytical Sensitivity

Molecular Assay Analytical Sensitivity and Linearity

The Limit of Blank (LoB), Limit of Detection (LoD), Lower Limit of Quantitation (LLoQ), linearity, and linear range were determined for each of the four markers of the Molecular Assay component of the Cologuard Plus test. A summary of the results of this study is in Table 2.

The study included a minimum of six days, two reagent lots, one instrument, three pooled patient samples, and six dilutions per sample. Samples were prepared using sDNA from de-identified patient samples from the Cologuard Plus process. Blank samples had no detected signal in the LQAS assay. Therefore, the LoD and LoQ values were established independent of the LoB measurement, defined as the concentration of DNA where 95% of runs are detected at or below that concentration. To show that blank samples result in limited signal, the data for all no-template controls included on the 18 LoD/LoQ plates (n=72) were evaluated. An LoB of 0 strands was confirmed for all 4 markers.

The LoD is the concentration corresponding to 95% detection probability. The concentration where the robust CV falls below 20% was considered the LoQ of molecular assay. Established LoD and LoQ values are listed in the table below.

The linearity and linear range study was conducted using two lots of reagents, one QuantStudio™ 5 Dx Real-Time PCR Instrument (QS5Dx) instrument and one operator. Two PCR plates were setup per reagent lot for a total of four plates. Two dilution series were prepared using two unique sample pools. The sample pools were prepared from spiked and unspiked pools of clinical samples diluted with blank diluent.

The linear range was determined as the lowest or highest point that provided results within the pre-specified allowable deviation from linearity (ADL). If any of those values fell below the LoD of the algorithm, the lower algorithm cutoff was claimed. The established linear range for each marker is outlined in the table below.

Table 2: Molecular Assay Analytical Sensitivity Characteristics Summary

Performance Characteristic	Result
Limit of Detection	LoD determined at level where 95% detection was met. 3 strands for LASS4 2 strands for PPP2R5C 2 strands for LRRC4 2 strands for ZDHHC1

Performance Characteristic	Result
Lower Limit of Quantitation	<p><20% CV at LLoQ concentrations</p> <p>25 strands for LASS4</p> <p>21 strands for PPP2R5C</p> <p>27 strands for LRRC4</p> <p>14 strands for ZDHHC1</p>
Linear Range	<p>9–1,380,384 strands for LASS4</p> <p>5–1,318,257 strands for PPP2R5C</p> <p>5–1,380,384 strands for LRRC4</p> <p>250–100,000 strands for ZDHHC1</p>

Based on the study data, the LoD claims of the Molecular Assay for LASS4, PPP2R5C, and LRRC4 are ≤ 5 strands and the LoD claim for ZDHHC1 is ≤ 251 strands. For the reference marker, ZDHHC1, samples with strand values $< 2.4 \log$ strands (approximately 251 strands) are called invalid. The value of 251 strands is not based on the anticipated LoD above or any other analytical performance characteristic of the molecular assay. Rather, this value is set to protect the miscall rate of the Cologuard Plus test as a whole testing system.

Hemoglobin Assay Analytical Sensitivity and Linearity

The LoB, LoD, LoQ, linearity, linear range, and Hook Effect were determined for the Hemoglobin Assay component of the Cologuard Plus test. A summary of the results is in 3 below.

The LoB, LoD, and LoQ study was conducted using two lots of reagents on a single instrument for four runs per reagent lot. Samples for the LoD and LoQ study were made from pools of stool samples with endogenous Hb and diluted to near LoD/LoQ levels using unique lots of Reconstitution Buffer. The 95th percentile of 80 replicates of Reconstitution Buffer for blank measurements was determined to be the LoB. LoD and LoQ were established with 64 replicates of four unique patient samples for each of the four concentration levels of 10.0, 7.5, 5.0, and 2.5 ng/mL. The concentration of Hb at which at least 95% of runs were above the LoB was determined as the LoD. LoQ was determined to be the concentration of Hb at which the CV is below 20%, is greater than or equal to LoB, and where LoD is not larger than LoQ.

The Hook Effect was assessed by testing four replicates of each of the 10 Hb concentration levels above, below, and spanning the anticipated quantitative range of one normal blood sample (HbA) and two hemoglobin variants (HbS and HbC). The mean values for all samples with concentrations above the upper algorithm limit of 1,000 ng/mL were compared for a decrease in signal. There was no bias from Hook Effect for Hb concentration of up to 100,000 ng/mL which included the 1 mg per gram of stool (10 μ g Hb input into assay) pre-specified in the acceptance criteria.

For the linearity study, two unique samples were diluted to 9 Hb concentration levels spanning the anticipated quantitative range of 10-1,000 ng/mL. A minimum of 4 replicates were tested for each sample and level. A linear regression function was fit for each sample. For each dilution level, the predicted value was compared to the mean of the repeats of that dilution. The difference was compared to a pre-specified allowable deviation from linearity (ADL). The anticipated quantitative range of 10–1,000 ng/mL was identified to be within the ADL.

Table 3: Hemoglobin Assay Analytical Sensitivity Characteristics Summary

Performance Characteristic	Analytical Sensitivity Study Result
Limit of Blank	2.0 ng/mL
Limit of Detection	2.9 ng/mL
Limit of Quantitation	2.9 ng/mL
Linearity	Linear range = 10–1,000 ng/mL
Hook Effect	No Hook Effect observed

C. Interfering Substances

This study evaluated the impact to the Cologuard Plus score due to interfering substances found in stool through ingestion or external application. Substances included common medications (such as antacids, antibiotics, anti-inflammatories, anti-fungal medications, pain relievers, decongestants, stool softeners, anti-diarrheal medications, and laxatives), urine, ethanol, cholesterol and fatty acids, vitamin C, iron, a mixture of fruits and vegetables, genomic DNA from common edible animals, hypomethylation agents, and DNA stabilization buffer in the Hb assay. High negative and low positive stool pools were prepared with and without the presence of these substances, and 10 replicates of each sample were tested in the molecular and hemoglobin assays. No meaningful amount of interference was detected for any interfering substances.

D. Specificity and Cross-Reactivity

This testing included an assessment of cross-reactivity of cancers and diseases other than colorectal cancer and analytical specificity of the methylation and hemoglobin markers that are detected by the Cologuard Plus test.

Non-Colorectal Cancers and Diseases

Specificity of the Cologuard Plus test 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 4: 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 369-395.⁵

Analytical Specificity

The Cologuard Plus Hemoglobin Assay is designed to detect patient-origin Hb in human stool and the Molecular Assay is designed to detect only fully methylated *LASS4*, *PPP2R5C*, *LRRC4* and *ZDHHC1* gene targets.

The Hemoglobin Assay was tested for cross-reactivity with Hb and Myoglobin (Mb) from animals that could be present in a human stool sample due to diet, and the Molecular Assay was tested with fully unmethylated DNA target sequences that are likely to be present as background in all patient samples. The Hemoglobin Assay had 10 replicates of each sample both unspiked and spiked with whole blood, Mb from meat extracts, or purified Mb from eight commonly eaten animal species (bovine, pig, turkey, chicken, trout, goat, rabbit, and sheep). The Molecular Assay had 45 replicates of each sample, both unspiked and spiked with synthetic, fully unmethylated DNA target sequences of each of the methylation markers combined into a single sample type.

Both marker-level and score-level assessments showed minimal cross-reactivity below the specified acceptance criteria to non-human Hb, non-human Mb for the Hemoglobin Assay and to unmethylated target sequences for the Molecular Assay.

E. Precision and Reproducibility

Precision and Reproducibility Study with Clinical Samples

This precision study examined reproducibility between three laboratory sites using a panel of four clinical samples. At each site, two operators performed testing for five non-consecutive days for a total of five assay runs. The sample panel consisted of six clinical samples prepared from de-identified patient specimens, and one control sample. The panel represented a range of pathologies including CRCs, APLs, and negatives with varying levels of marker signals and Cologuard Plus scores representing a wide range of test results including samples close to the algorithm cutoff (see Table 5).

Table 5: Reproducibility and Precision (Sample Panel Overview)

Sample Type	Pathology Type	Sample Matrix	Expected Result	Replicates per Run	Replicates per Site	Replicates Across Sites
High CRC Stool	CRC Stage III	Stool	Positive	6	30	90
Low CRC Stool	CRC Stage I	Stool	Positive	6	30	90
High APL Stool	Advanced Adenoma	Stool	Positive	6	30	90
Low APL Stool (C95)	Advanced Adenoma	Stool	Positive	6	30	90

High Negative Stool (C5)	Non-advanced Adenoma	Stool	Negative	6	30	90
Low Negative Stool	Negative	Stool	Negative	6	30	90
Low Positive Control	NA	Control	Positive	6	30	90

Percent agreement values were calculated between observed and expected Cologuard Plus results, yielding 100% agreement for positive samples, 96.6% agreement for negative samples, and 99.0% overall agreement across all samples. The lower 95% confidence limit for overall percent agreement was greater than 95% for all samples. Precision also exceeded 95% for all laboratory sites (see Table 6). In the design for this study, site was confounded with operator and instrument, and run was confounded with day.

Table 6: Percent Agreement by Site

	Percent Agreement (%)	Lower 95% CI (%)
Overall	99.0	98.1
Positive	100.0	99.3
Negative	96.6	93.5
Site 1	99.0	97.0
Site 2	98.1	95.7
Site 3	100.0	98.6

Four sample types — the High Negative Stool (C5), Low APL Stool (C95), High APL Stool, and Low Positive Control had mean Hb concentrations less than 300 ng/mL and mean AvgMDM values greater than 0 and thus were subject to the SD acceptance criterion. These samples showed a maximum upper 95% CI SD of 39 (see Table 7).

Table 7: SD of Cologuard Plus Score and Upper 95% CI of SD

Sample	N	Mean	SD	Upper 95% CI
High CRC Stool	89	2031	64	73
Low CRC Stool	89	1169	42	48
High APL Stool	90	371	20	23
Low APL Stool (C95)	89	102	22	26
High Negative Stool (C5)	90	-56	34	39

Sample	N	Mean	SD	Upper 95% CI
Low Negative Stool	89	-382	76	86
Low Positive Control	90	214	23	26

Precision and Reproducibility with Contrived Samples

The precision study was supplemented with contrived samples to evaluate the examined reproducibility between three laboratory sites, using a minimum of two operator groups per site, and two instrument groups per site. Testing was performed across 22 assay runs at each site using five contrived stool samples. All contrived samples including C5 and C95 samples included all markers. The C5 sample consisted of solely endogenous target for markers LASS4, PPP2R5C, and LRRC4, while for marker ZDHHC1, target consisted of both endogenous and synthetic sources. The C95 sample consisted of solely endogenous target for markers LASS4, and LRRC4, while for markers PPP2R5C and ZDHHC1, target consisted of both endogenous and synthetic sources. The high positive and mid positive samples were designed to target the mean marker levels observed in the algorithm study from late stage (Stage III) and early stage (Stage I) CRC, respectively. Marker levels from the algorithm study from advanced precancerous lesions (APL) and non-advanced adenoma samples were used as the basis of the C95 and C5 samples, respectively. Three synthetic controls were prepared with varying levels of marker signals and Cologuard Plus scores representing a wide range of test results including samples close to the algorithm cutoff (see Table 8). A contrived specimen functional equivalency (CSFC) study was performed to demonstrate the equivalent performance between clinical and contrived samples.

Table 8: Precision and Reproducibility Sample Panel Overview

Sample Type	Pathology Type	DNA Sample Matrix	Hb Sample Matrix	Expected Result	Replicates per Run	Replicates per Site	Replicates Across Sites
High positive stool	CRC Stage III	Stool	Stool	Positive	6	132	396
Mid positive stool	CRC Stage I	Stool	Stool	Positive	6	132	396
Negative stool	Negative	Stool	Stool	Negative	6	132	396
C5 ^a	Non-advanced Adenoma	Stool	Stool	Negative	6	132	396
C95 ^b	Advanced Adenoma	Stool	Stool	Positive	6	132	396
High Positive Control	NA	Buffer	Buffer	Positive	5	110	330

Sample Type	Pathology Type	DNA Sample Matrix	Hb Sample Matrix	Expected Result	Replicates per Run	Replicates per Site	Replicates Across Sites
Low Positive Control	NA	Buffer	Buffer	Positive	5	110	330
Negative Control	NA	Buffer	Buffer	Negative	4	88	264

- a 5% of pool replicates are expected to have a positive test result due to measurement error.
- b 95% of pool replicates are expected to have a positive test result and 5% are expected to have a negative result due to measurement error.

Percent agreement values were calculated between observed and expected Cologuard Plus results, yielding 100% agreement for positive samples, 98.9% agreement for negative samples, and 99.6% agreement for the pooled results. The lower 95% confidence limit for call concordance exceeded 95% for all samples. Precision exceeded 95% for all operators, instruments, and laboratory sites. (see Table 9).

Table 9: Call Concordance Between Sites, Operators, and Instruments

Comparison	Pairs of samples	Sample pairs match	Agreement	Lower 95% CI
Site 2 vs Site 3	959	950	99.1%	98.2%
Site 2 vs Site 1	949	941	99.2%	98.3%
Site 3 vs Site 1	949	946	99.7%	99.1%
Site 1 Operators	466	463	99.4%	98.1%
Site 2 Operators	475	467	98.3%	96.7%
Site 3 Operators	475	474	99.8%	98.8%
Site 1 Instruments	466	463	99.4%	98.1%
Site 2 Instruments	475	467	98.3%	96.7%
Site 3 Instruments	475	474	99.8%	98.8%

Four sample types with mean Hb concentrations less than 300 ng/mL and mean AvgMDM (median weighted average of the reference-normalized, standardized methylation marker DNA concentrations) values greater than 0 showed a maximum upper 95% CI of 39. (see Table 10).

Table 10: SD of Cologuard Plus Score and Upper 95% CI of SD

Sample	N	Mean Score	SD Score	Upper 95% CI
C5	390	-69	36	39

Sample	N	Mean Score	SD Score	Upper 95% CI
C95	394	94	24	26
High Positive Control	328	1113	24	26
High positive stool	392	2050	81	87
Low Positive Control	329	225	22	23
Mid positive stool	395	1369	49	53
Negative Control	263	-430	23	25
Negative stool	394	-469	52	55

The lot-to-lot reproducibility of the molecular and hemoglobin (Hb) assay reagents was assessed to demonstrate that the 95% lower confidence limit on the percent agreement between reagent lots was $\geq 95\%$. A single site study was performed with three reagent lots made with unique raw materials where possible and three lots of consumables. Three runs per reagent lot were completed for each assay. The sample panel used in this study included a panel of five contrived samples and three control samples, possessing varying levels of methylated DNA markers (MDMs) and Hb concentration to provide a range of Cologuard Plus scores. The targeted scores were chosen to mimic clinical specimens representative of the intended use population and to encompass samples with scores near the algorithm cut-off. Table 11 below outlines the number of replicates analyzed for each sample type included in this study.

Table 11: Summary of Lot-to-lot Reproducibility

Sample Type	Pathology Type	DNA Sample Matrix	Hb Sample Matrix	Expected Result	Replicates per Run	Replicates per Reagent Lot	Replicates Across Lots
High positive stool	CRC Stage III	Stool	Stool	Positive	6	18	54
Mid positive stool	CRC Stage I	Stool	Stool	Positive	6	18	54
Negative stool	Negative	Stool	Stool	Negative	6	18	54
C5	Non-advanced Adenoma	Stool	Stool	Negative	6	18	54

Sample Type	Pathology Type	DNA Sample Matrix	Hb Sample Matrix	Expected Result	Replicates per Run	Replicates per Reagent Lot	Replicates Across Lots
C95	Advanced Adenoma	Stool	Stool	Positive	6	18	54
High Positive Control	NA	Buffer	Buffer	Positive	5	15	45
Low Positive Control	NA	Buffer	Buffer	Positive	5	15	45
Negative Control	NA	Buffer	Buffer	Negative	4	12	36

Four samples had Hb < 300 ng/mL and AvgMDM > 0, and thus were subject to the SD acceptance criterion. All results passed, as outlined in Table 12.

Table 12: SDs of Relevant Sample Types

Sample Type	N	Mean Score	SD Score	Upper 95% CI
C5	53	-83	38	47
C95	54	94	23	28
High Positive Control	44	1091	26	33
High Positive Stool	54	2083	57	70
Low Positive Control	45	218	23	29
Mid Positive Stool	52	1393	53	66
Negative Control	36	-429	29	38
Negative Stool	54	-492	58	72

Additionally, all samples were found to have 100% concordance with the expected calls per sample type as shown in Table 13 below.

Table 13: Concordance Values

Condition	Concordance	Lower 95% CI	Specification	N
Total	100%	99.1%	≥95%	392

Condition	Concordance	Lower 95% CI	Specification	N
Lot 1 vs Lot 2	100%	98.6%	≥95%	262
Lot 1 vs Lot 3	100%	98.6%	≥95%	260
Lot 2 vs Lot 3	100%	98.6%	≥95%	260

Specimen Reproducibility

Clinical specimen reproducibility study examined assay performance and call concordance with stool samples of known pathology (10 CRC, 10 APL, and 10 Negative) for 30 individual subjects (Table 14). For each individual subject, three stool homogenates aliquots from the same whole stool collection kit and three aliquots of fecal occult hemoglobin from the same FIT tube were tested through the Cologuard Plus workflow.

Samples were selected to represent a range of disease states, a range of molecular marker and Hb values, and a range of Cologuard Plus scores, including some near the assay cut-off.

Table 14: Sample Panel Results

Subject ID	Pathology	Category	Stage	Mean Score	Standard Deviation Score	CV Score	N Valid	N Pos	N Neg	% Concordant
150HTWD	CRC	1	Stage I	1502	15	1	3	3	0	100%
150HZO8	CRC	1	Stage I	83	58	70	3	3	0	100%
150R0D7	CRC	1	Stage I	308	19	6	3	3	0	100%
150R0K5	CRC	1	Stage I	807	18	2	3	3	0	100%
160AVST	CRC	1	Stage I	935	15	2	3	3	0	100%
160C1OK	CRC	1	Stage I	1394	33	2	3	3	0	100%
170C3T1	CRC	1	Stage I	1180	26	2	3	3	0	100%
150GBF1	CRC	1	Stage II	1755	10	1	3	3	0	100%
150SBSC	CRC	1	Stage II	1221	2	0	3	3	0	100%
150KH7K	CRC	1	Stage III	1096	23	2	3	3	0	100%
150YAOD	APL	2.1	N/A	286	43	15	3	3	0	100%
150HL8Q	APL	2.2	N/A	475	14	3	3	3	0	100%
150VG3M	APL	2.2	N/A	779	14	2	3	3	0	100%
150XXKZ	APL	2.2	N/A	254	22	9	3	3	0	100%
1602UA0	APL	2.2	N/A	335	6	2	3	3	0	100%
150FETS	APL	2.3	N/A	95	18	19	3	3	0	100%
150LF27	APL	2.3	N/A	1532	70	5	3	3	0	100%
150S16G	APL	2.3	N/A	986	36	4	3	3	0	100%
150ZWCA	APL	2.3	N/A	343	21	6	3	3	0	100%
150NCZB	APL	2.4	N/A	184	5	3	3	3	0	100%

Subject ID	Pathology	Category	Stage	Mean Score	Standard Deviation Score	CV Score	N Valid	N Pos	N Neg	% Concordant
150S18F	Normal	3	N/A	-86	78	-90	3	0	3	100%
150IG05	Normal	4	N/A	-26	102	N/A*	3	1	2	67%
16028XZ	Normal	5	N/A	-312	36	-12	3	0	3	100%
150IFZ9	Normal	6.1	N/A	-158	50	-32	3	0	3	100%
150L83S	Normal	6.1	N/A	6	21	N/A*	3	2	1	33%
15011VM	Normal	6.1	N/A	-68	32	-47	3	0	3	100%
150JZBF	Normal	6.2	N/A	-666	175	-26	3	0	3	100%
150KTLZ	Normal	6.2	N/A	-245	40	-16	3	0	3	100%
150UCH2	Normal	6.2	N/A	-388	54	-14	3	0	3	100%
150A3Y	Normal	6.2	N/A	-464	34	-7	3	0	3	100%

*CVs not calculated as score replicates span zero.

Call concordance between the three aliquots was 100% for all but two samples, both of which were normal (negative) samples near the clinical decision point.

F. **Robustness**

This study evaluated the robustness of the Cologuard Plus test in response to variation in specific steps in the molecular and hemoglobin assay procedures. Specifically, several steps in the Cologuard Plus workflow require user handling, such as sample handling, reagent aspiration, and reagent dispensing, and variability in these steps could affect the test result. Testing was performed using three operators for the molecular testing and two operators for the hemoglobin testing. One set of instrumentation was used for each test factor, and a single reagent lot was used for the study.

Factors tested in the Molecular Assay included the following:

- Variations in volume adjustment of clarified stool supernatant and amount of Capture Beads and Capture Wash added.
- Time delay in addition of Capture Beads, Capture Solution, and Capture Wash
- Time delay to start capture incubation after addition of Capture Beads and Capture Solution while on bench and while in incubator.
- Time delay to load LQAS reagents onto STARlet, and to load unsealed LQAS plate into QS5Dx.

Factors tested in the Hemoglobin Assay included the following:

- Variation in volume of Reconstitution Buffer addition to calibrators and controls.
- Time delay to read plate on plate reader.

The results of this study assessing variation at specific steps requiring user handling showed that for all the robustness factors, the Cologuard Plus scores for the test condition were within the pre-specified acceptance criteria (the mean Cologuard Plus score for each sample was +/-80 units from the mean Cologuard Plus score for the standard condition).

G. Carry-over and Cross-contamination

This study examined the impact of carry-over and cross-contamination of the Cologuard Plus workflow on assay results. Testing was initiated with a single assay run with high negative samples (control stage), followed by five assay runs comprised of alternating negative and high positive samples prepared in a checkerboard sequence (test stage). Assay results were used to calculate a Cologuard Plus score and qualitative test result. Positive test rates of the negative samples were compared between the test and control stage to test for non-inferiority as a measure of clinical significance due to contamination. Results showed no difference in the positive test rate between the test and control stages, with no incorrect calls (the upper bound U of a 97.5% one-sided confidence interval for the difference in Test-Control positive rate is < 10.0%). Cross-contamination was also examined per marker, except for reference marker *ZDHHCl*, by comparing the difference in mean marker signal observed in the control stage and test stage for the negative samples. Results from the analysis demonstrated an acceptably low level of carry-over and cross-contamination in the Cologuard Plus workflow. The upper bound of the difference between the test and control stages was less than 1% of the high positive mean.

H. Sample Stability

Testing was performed to establish the in-process specimen stability at various stopping points in the Cologuard Plus workflow. These included:

- Stability of DNA hybridized to capture probes conjugated to magnetic beads (captured DNA) at room temperature (0, 3, 6, 8 and 9 hours)
- Stability of eluted, bisulfite-converted DNA at 2-8°C (1, 2, 3, 4 and 5 days)
- Stability of PVPP treated (clarified) stool supernatant at room temperature (0, 2, 4, 6 and 7 hours)
- Stability of thawed stool homogenate at room temperature (0, 2, 4, 6 and 7 hours)
- Stability of the Hb tube at 2-8°C (0, 3, 7, 10, 14 and 15 days)
- Stability of the Hb tube at room temperature (0, 6, 12, 18, 24 and 25 hours)

Samples for the Molecular Assay included a negative synthetic control and a positive pooled clinical sample. For the Hemoglobin Assay, samples included pooled clinical samples with low and high Hb levels. Greater than or equal to 12 replicates were tested at each time point, and stability was evaluated by using linear regression to model the effect of time on the Cologuard Plus score and / or marker concentrations.

All conditions met the pre-specified acceptance criteria at all time points, supporting a claim of the penultimate time point for each condition tested.

I. Reagent Stability

In-Use Reagent Stability

In-use reagent stability testing was performed to establish the stability recommendations for multiple-use reagents and controls once reagent containers had been opened and for on-deck automation reagents once they were poured into troughs or placed on-deck prior to run initiation. Testing was performed separately for the molecular and hemoglobin portions of the Cologuard Plus test.

The multiple-use reagents and controls were tested at 7 time points (0, 31, 41, 62, 72, 93 and 100 days). The on-deck automation reagents were tested at 5 time points (0, 2, 4, 6 and 7 hours). For all reagents, a total of 13 replicates per sample type were run at each time point. To determine stability, linear regression was used to model the effect of time on the Cologuard Plus scores or marker concentrations.

All reagent groups met the pre-specified acceptance criteria for stability at all time points, supporting a claim of 3 months in-use stability for the multiple-use reagents and controls, and 6 hours for the on-deck automation reagents.

Real-Time Reagent Stability

A real-time stability study is being run to establish the stability of the Cologuard Plus test Molecular Assay reagents and DNA controls, as well as the Cologuard Plus test Hemoglobin Assay reagents and controls. The study plans to evaluate the functional performance of three reagent lots over the course of 27 months, with the goal of establishing a minimum 24-month stability of the on-test reagents and controls. The following stability metrics will be measured: Cologuard Plus Score, AvgMDM and *ZDHHC1* log strands for the Molecular Assay Reagents; Cologuard Plus score and hemoglobin concentration for the Hemoglobin Assay Reagents; log strands of the DNA markers for the Molecular Assay DNA Controls; and hemoglobin concentration for the Hemoglobin Assay Controls. Interim analyses support a stability duration of at least six months for all reagents and controls.

Freeze/Thaw Reagent Stability

A freeze/thaw stability study was performed to evaluate the stability of LQAS reagents stored at -20°C. Four conditions were evaluated in the study: 0, 2, 4, and 6 freeze/thaw cycles. Additionally, two sample types were tested: synthetic target in a run control matrix, intended to provide a negative Cologuard Plus score, and endogenous target in a stool matrix, intended to provide a positive Cologuard Plus score. To determine stability, linear regression was used to model the effect of the number of freeze/thaw cycles on the Cologuard Plus score.

All reagent groups met the pre-specified acceptance criteria for stability at all time points, demonstrating that the LQAS reagents are stable for up to four freeze/thaw cycles.

Shipping Stability

Testing was performed to evaluate the stability of the Hb and whole stool samples under shipping stress conditions. The Hemoglobin and Molecular Assay samples were subjected to ship stress conditions, and evaluated for a period of 0, 3, 4, 5, 6 and 7 days, and 0, 1, 3, 5, 8 and 9 days, respectively. The Hb study used pooled clinical samples with

low, mid, and high Hb levels, and evaluated 16 replicates at each time point. The DNA study used a panel of 20 positive and negative clinical samples and evaluated 3 replicates at each time point. Stability was assessed by using linear regression to model the effect of time on the Cologuard Plus score and / or marker concentrations.

The results of the studies demonstrated all conditions met the pre-specified acceptance criteria at all time points, supporting a claim of 6 days for the Hb sample, and 8 days for the DNA sample.

J. Collection Kit Testing

The following studies were performed for the Cologuard Collection Kit in the original Cologuard test:

- Real-time reagent stability testing to establish the shelf-life of the collection kit reagents.
- Shipping integrity testing to demonstrate that the collection kit withstands the typical stresses of shipment between the kit supplier, distribution center, patient, and clinical laboratory.
- Human factors testing to demonstrate that patients can successfully use the collection kit in an at-home environment.

The kit components and collection process are identical for both Cologuard and Cologuard Plus tests.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of the Cologuard Plus test was evaluated in the prospective, cross-sectional, multi-center, pivotal study, named BLUE-C, to generate data to support the safety and effectiveness of Cologuard Plus for CRC and APL screening in the US.⁶ The primary objective was to assess the sensitivity for CRC detection and specificity of the Cologuard Plus test when compared to colonoscopy results.

The secondary objectives were to assess the sensitivity of the Cologuard Plus test for APL detection; compare the sensitivities for CRC and APL detection of Cologuard Plus test to a commercially available FIT; and evaluate the specificity of Cologuard Plus test for participants with no colorectal neoplastic findings.

A summary of the clinical study is presented below.

A. Study Design

The BLUE-C study enrolled a total of 26,758 participants at 186 sites in the United States (ClinicalTrials.gov, Trial Registration ID: NCT04144738). Participants were considered enrolled if they met all eligibility criteria during screening and provided written informed consent. Participants were provided with a stool collection kit, which included collection materials for Cologuard Plus, a commercial FIT, and sample collection instructions to

complete the stool collection prior to bowel preparation for the colonoscopy procedure. To evaluate the performance of Cologuard Plus, the test result was compared to the colonoscopy result and histopathological information collected for tissue removed during colonoscopy and, if applicable, any follow-up procedures. Colorectal lesions identified during colonoscopy were categorized based on the most clinically significant lesion present (Index Lesion), as indicated in the Table 15. The American Joint Committee on Cancer (AJCC) Staging System, 8th edition, was used for recording CRC stages.⁴

The study additionally compared the performance of the Cologuard Plus test with a commercially available Fecal Immunochemical Test (FIT) (Polymedco OC-Auto[®] Micro 80 iFOB test) for CRC and APL detection.

Table 15: 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 lesions with dysplasia (SSLDs); Traditional serrated adenoma (TSA); Conventional adenomas with serrated architecture; Sessile serrated lesions, ≥ 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.

Investigators and/or colonoscopists were blinded to all Cologuard Plus test and FIT results. Individuals conducting the Cologuard Plus test laboratory testing were blinded to all clinical data and to the results of the FIT. Cologuard Plus performance and FIT

performance were assessed and compared to evaluate whether the study objectives were met.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the BLUE-C study was limited to subjects who met the following inclusion criteria:

- Participant is male or female, 40* years of age or older.
- Participant presents for a screening colonoscopy per standard of care.
- Participant has no symptoms or signs that require immediate, or near term, referral for diagnostic or therapeutic colonoscopy.
- Participant understands the study procedures and can provide informed consent to participate in the study and authorization for release of relevant Protected Health Information (PHI) to the study Investigator.

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

- A personal history of colorectal cancer or advanced precancerous lesions.
- A positive result from another colorectal cancer 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
- Personal history of any of the following high-risk conditions for colorectal cancer:
 - 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.
- Participant has undergone a colonoscopy within the previous 9 years, with the exception of a failed colonoscopy due to poor bowel preparation. Failed colonoscopy must have been within the past year and without therapeutic intervention.
- Participant has had overt rectal bleeding within the previous 30 days.
- Participant has any condition that in the opinion of the Investigator should preclude participation in the study.

* The enrolled patients who were between 40-44 years of age have been excluded from the data analysis.

2. Clinical Performance Measures

The primary analysis population consisted of all enrolled participants with a valid Cologuard Plus test result, an evaluable colonoscopy, and meeting all study eligibility criteria. In addition to the study enrollment eligibility criteria, the primary analysis

population excluded participants with a first-degree relative with CRC diagnosed at any age, as well as participants under the age of 45 years.

Primary Endpoints

The two pre-specified primary endpoint hypotheses were:

- (1) to test if the Cologuard Plus test sensitivity for CRC rejects the 75% null hypothesis, and
- (2) to test if the Cologuard Plus test specificity for participants without advanced neoplasia (CRC or APL) rejects the 85.9% null hypothesis.

Each primary hypothesis was evaluated using a one-sided exact binomial test at the 2.5% significance level, corresponding to requiring the one-sided 97.5% exact binomial confidence bound (or, equivalently, the lower bound of the 2-sided 95% exact confidence interval (CI)) to be greater than the null hypothesis value. Both primary null hypotheses needed to be rejected for the study to be considered successful.

Secondary Endpoints

The four secondary endpoint hypotheses were:

- (1) to test if the Cologuard Plus test sensitivity for participants with APL findings rejects the 38.9% null hypothesis,
- (2) to test if the Cologuard Plus test sensitivity for CRC detection is superior to that of a commercially available FIT,
- (3) to test if the Cologuard Plus test sensitivity for APL detection is superior to that of a commercially available FIT, and
- (4) to test if Cologuard Plus test specificity for participants with no colorectal neoplastic findings rejects an 87.5% null hypothesis.

Secondary hypotheses (1) and (4), were evaluated using a one-sided exact binomial test at 2.5% significance level, corresponding to requiring the one-sided 97.5% exact binomial confidence bound (or, equivalently, the lower bound of the 2-sided 95% exact CI) to be greater than the null hypothesis value.

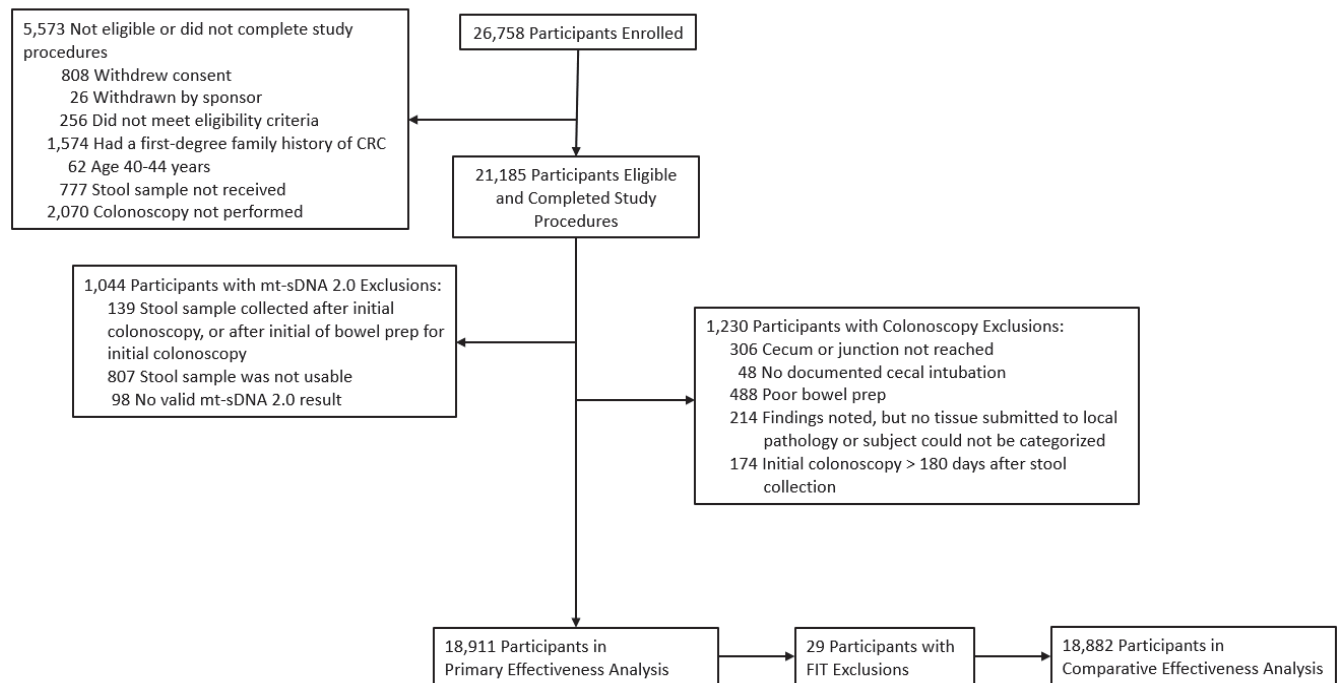
The head-to-head comparisons with the commercial FIT were performed using exact McNemar's tests for paired proportions at the one-sided 2.5% significance level.

B. Accountability of PMA Cohort

Of the total 26,758 participants enrolled in the study, 18,911 were included in the primary effectiveness population and 18,882 in the comparative effectiveness population. 5,573 participants were not included in the primary analysis population due not meeting analysis inclusion-exclusion criteria or not completing all study procedures. Of the remaining 21,185 participants who completed study procedures, 1,044 had exclusions related to stool sample or testing (139 stool samples collected after initial colonoscopy or

bowel preparation, 807 stool samples received unusable per protocol, 98 invalid Cologuard Plus test results), and 1,230 were excluded for lack of an evaluable colonoscopy, resulting in 18,911 participants in the primary effectiveness population, of which 29 did not have a usable and valid FIT result, resulting in 18,882 participants in the comparative effectiveness population (Figure 1).

Figure 1: Flow Chart of Participants



C. Study Population Demographics and Baseline Parameters

The distribution of race and ethnicity among BLUE-C participants included in the primary effectiveness population closely mirrored that of the United States population, as reported in the 2020 Census results. The average age of participants was 63.0 years, and 53.1% of participants were female. The race and ethnicity distribution of participants was 59.7% White, not Hispanic or Latino; 16.4% Hispanic or Latino; 13.4% Black or African American, not Hispanic or Latino; and 9.0% Asian, not Hispanic or Latino. There was a small percentage of other race and ethnic participants including American Indian and Native Hawaiian participants included in the study. Average BMI was 29.5 kg/m² and 63.6% participants had never smoked. 32.0% of the participants had had a colonoscopy (>9 years prior to enrollment) in their lifetime and 3.8% had a prior Cologuard test (Table 16).

Table 16: Performance characteristics by demographic factors and baseline characteristics

Parameter Statistic	All Subjects (N=18,911)
Age (years)	
n	18,911
Mean (SD)	63.0 (7.2)
Median	64
Min, Max	45, 86
Age, n (%)	
45-49 years	289 (1.5)
50-54 years	1,535 (8.1)
55-59 years	4,550 (24.1)
60-64 years	3,551 (18.8)
65-69 years	5,488 (29.0)
70-74 years	2,494 (13.2)
≥75 years	1,004 (5.3)
Age, n (%)	
<55 years	1,824 (9.6)
55-64 years	8,101 (42.8)
≥65 years	8,986 (47.5)
Sex, n (%)	
Male	8,876 (46.9)
Female	10,035 (53.1)
Race, n (%)	
White	14,083 (74.5)
Black or African American	2,607 (13.8)
Asian	1,714 (9.1)
American Indian or Alaskan Native	82 (0.4)
Native Hawaiian or Other Pacific Islander	31 (0.2)
Multiracial	89 (0.5)

Parameter Statistic	All Subjects (N=18,911)
Other	286 (1.5)
Missing	19
Ethnicity, n (%)	
Hispanic or Latino	3,094 (16.4)
Not Hispanic or Latino	15,689 (83.0)
Unknown	127 (0.7)
Missing	1
Race/Ethnicity, n (%)	
White, Not Hispanic or Latino	11,286 (59.7)
Hispanic or Latino	3,094 (16.4)
Black or African American, Not Hispanic or Latino	2,532 (13.4)
Asian, Not Hispanic or Latino	1,704 (9.0)
American Indian or Alaskan Native, Not Hispanic or Latino	67 (0.4)
Native Hawaiian or Other Pacific Islander, Not Hispanic or Latino	23 (0.1)
Multiracial, Not Hispanic or Latino	77 (0.4)
Other, Not Hispanic or Latino	116 (0.6)
Missing	12
BMI (kg/m ²) at Baseline	
n	18,906
Mean (SD)	29.5 (6.4)
Median	28.6
Min, Max	13.0, 69.2
Tobacco History, n (%)	
Never Smoked	12,019 (63.6)
Former Smoker	4,612 (24.4)
Current Smoker	2,280 (12.1)

Parameter Statistic	All Subjects (N=18,911)
CRC Screening History (non-colonoscopy), n (%)	
Yes	2,141 (11.3)
CT Colonography	14 (0.1)
Flexible Sigmoidoscopy	71 (0.4)
gFOBT	773 (4.1)
FIT	593 (3.1)
Multi-target stool DNA test	717 (3.8)
No	16,770 (88.7)
Prior Colonoscopy, n (%)	
Yes	6,054 (32.0)
No	12,857 (68.0)
Note 1: Column percentages exclude missing values.	

D. Safety and Effectiveness Results

1. Safety Results

Adverse effects that occurred in the PMA clinical study

Due to the design of the study and nature of the stool collection process, serious adverse events caused by or related to the stool collection procedure were not anticipated. During the BLUE-C clinical study, no adverse events related to stool collection were reported.

The Cologuard Plus test has the risk of a false test result (i.e., a false positive or a false negative result). All positive test results should be followed by a colonoscopy. False positive Cologuard Plus results could lead to an increased number of colonoscopies and associated adverse events related to the colonoscopy procedure. A false negative Cologuard Plus result could lead to a colorectal cancer or precancerous lesions remaining undetected.

2. Effectiveness Results

Primary Effectiveness Evaluation

Data was analyzed for 18,911 participants meeting criteria for inclusion in the primary effectiveness population (Table 17).

Table 17: Summary of Cologuard Plus Test Performance

Colonoscopy / Histopathology	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 positive predictive value (PPV) of the Cologuard Plus test was 3.2% for CRC and 34.0% for APL. Among 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 negative predictive value (NPV) for CRC of the Cologuard Plus test was 99.98%, with only 0.02% of participants with a negative test result having CRC (Table 18).

Table 18: Index Lesion Categorization by 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

Secondary Effectiveness Evaluation

In the comparative effectiveness population, sensitivity for CRC was greater for the Cologuard Plus test compared to independent 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 Cologuard Plus. Sensitivity for APL was greater for the Cologuard Plus test compared to independent 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 Cologuard Plus.

CRC and APL sensitivity was consistently higher for the Cologuard Plus test compared to independent FIT across cancer stages, lesion sizes, lesion locations, and APL subtypes as shown in following tables (Table 19-20).

Table 19: Cologuard Plus CRC Sensitivity by Colonoscopy Categories, Compared to independent FIT CRC Sensitivity

CRC Subgroup	Cologuard Plus CRC Sensitivity	independent FIT CRC Sensitivity
Index Lesion Size, % (95% CI); n/N		
<5 mm	100.0 (2.5-100.0); 1/1	100.0 (2.5-100.0); 1/1
5–9 mm	100.0 (2.5-100.0); 1/1	100.0 (2.5-100.0); 1/1
10–19 mm	87.5 (47.3-99.7); 7/8	62.5 (24.5-91.5); 5/8
20–29 mm	92.3 (64.0-99.8); 12/13	61.5 (31.6-86.1); 8/13
≥30 mm	96.8 (88.8-99.6); 60/62	72.6 (59.8-83.1); 45/62
Index Lesion Location, % (95% CI); n/N		
Proximal	93.5 (78.6-99.2); 29/31	61.3 (42.2-78.2); 19/31
Distal	93.8 (79.2-99.2); 30/32	78.1 (60.0-90.7); 25/32
Rectal	100.0 (84.6-100.0); 22/22	72.7 (49.8-89.3); 16/22
CRC Stage, % (95% CI); n/N		
I	88.0 (68.8-97.5); 22/25	56.0 (34.9-75.6); 14/25
II	92.9 (66.1-99.8); 13/14	78.6 (49.2-95.3); 11/14
III	100.0 (88.4-100.0); 30/30	73.3 (54.1-87.7); 22/30
IV	100.0 (73.5-100.0); 12/12	83.3 (51.6-97.9); 10/12
X	100.0 (39.8-100.0); 4/4	75.0 (19.4-99.4); 3/4
Stage I-III combined	94.2 (85.8-98.4); 65/69	68.1 (55.8-78.8); 47/69

Table 20: Cologuard Plus 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*				
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%

APL Subgroup	Cologuard Plus n/N	Cologuard Plus Sensitivity	FIT n/N	FIT Sensitivity
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%

*Refer to Table 15: Participant Categorization Based on Histopathologic Diagnosis of the Index Lesion for APL subcategory definitions.

Results for CRC sensitivity, APL sensitivity, and specificity were consistent with the primary and secondary endpoint results in age-weighted estimation based on the age distribution of the US Population, multiple imputation for missing test results, and analysis of all available data.

Subgroup Analysis

The following baseline characteristics were evaluated for potential association with safety and effectiveness outcomes: sex, age, and race/ethnicity (Table 21).

Table 21: Cologuard Plus Performance by Subgroup

Subgroup	CRC Sensitivity %; n/N	APL Sensitivity %; n/N	Specificity for Category 3–6 %; n/N
Sex			

Subgroup	CRC Sensitivity %; n/N	APL Sensitivity %; n/N	Specificity for Category 3-6 %; n/N
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

The subgroup analysis showed that 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.

Overall, the results of the BLUE-C study demonstrate the safety and effectiveness of the Cologuard Plus test as a non-invasive, stool-based method for use in average risk adults for colorectal cancer screening.

3. Pediatric Extrapolation

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

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 828 investigators, including both primary and sub-investigators, of which none were full-time or part-time employees of the sponsor and one had disclosable financial interests / arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: None
- Significant payment of other sorts: None
- Proprietary interest in the product tested held by the investigator: One
- Significant equity interest held by investigator in sponsor of covered study: None

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

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Data from analytical studies demonstrated acceptable analytical performance of Cologuard Plus.

The pivotal clinical study 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.

Overall, the pivotal clinical study demonstrated that the Cologuard Plus test met both primary and secondary endpoints for sensitivity and specificity of the study.

B. Safety Conclusions

Risks associated with the collection of the stool sample necessary for the Cologuard Plus test are minimal. During the pivotal clinical study, no adverse events related to stool collection were reported.

With respect to the Cologuard Plus test itself, as with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. The primary risk associated with the Cologuard Plus test is a false test result (i.e., a false positive or a false negative result). Since all positive test results should be followed by colonoscopy, false positive results may lead to patients being referred to colonoscopy unnecessarily. Adverse events commonly associated with colonoscopy include abdominal discomfort and bowel irregularity post-procedure. Rare adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to the sedation resulting in respiratory and/or cardiac events, stroke and death.

In the instance of a false negative result, there is a possibility that a case of CRC or APL could go undetected, which could lead individuals with CRC or AA to forgo other recommended screening procedures such as colonoscopy.

C. Benefit-Risk Determination

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

The probable benefits of the Cologuard Plus device are based on data collected in the BLUE-C study, which was a prospective, cross-sectional, multi-center, pivotal study for the use of the Cologuard Plus device. Of the total 26,758 participants enrolled in the study, 18,911 average risk patients were included in the Primary Effectiveness Population and 18,882 average risk patients were included in the Comparative Effectiveness Population. For the Comparative Effectiveness study, the performance of the Cologuard Plus™ device was compared with a commercially available Fecal Immunochemical Test (FIT) (Polymedco OC-Auto® Micro 80 iFOB test) for CRC and APL detection.

The study conducted demonstrated probable benefit for CRC detection and detection of advanced precancerous lesions (APL). The sensitivity for CRC was observed to be 95.3% (81/85, 2-sided 95% CI: 88.4-98.7%) and the sensitivity for APL was 43.3% (849/1962, 2-sided 95% CI: 41.1-45.5%). The specificity was 90.7% (849/1962, 95% CI: 90.3%-91.1%). The positive predictive value (PPV) of the Cologuard Plus™ test was 3.2% for CRC and 34.0% for APL. Among 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 negative predictive value (NPV) for CRC of the Cologuard Plus™ test was 99.98%, with only 0.02% of participants with a negative test result having CRC. However, the NPV of the device for Advanced Neoplasia (CRC plus APL) was 93.18%, due predominantly to the APLs not detected by this device. The study additionally compared the performance of the Cologuard Plus™ test with a commercially available Fecal Immunochemical Test (FIT) (Polymedco OC-Auto® Micro 80 iFOB test) for CRC and APL detection, and generally demonstrated better performance than this FIT test for the sensitivity for these lesions.

To take a deeper look, the performance for subgroups was also examined. The sensitivity for Stage I, II and III CRC were 88.0% (22/25), 92.9% (13/14) and 100% (30/30), respectively, demonstrating a reasonable benefit in detection of early-stage CRC, for a non-invasive device. The sensitivity for APLs with high grade dysplasia was 73.6% (78/106), tubulovillous adenomas of any size was 54.8% (269/491) and serrated precancerous lesions was 49.4% (116/235). Given the totality of the data

provided for in the study, the Cologuard Plus™ device is deemed to have significant probable benefit in the detection of CRC, APL, with an acceptable level of specificity. Additional probable benefits of this test, include that it is noninvasive and has the potential to detect CRC/APL lesions earlier, than without screening, which may translate to better outcomes for patients. Despite the data provided, the probability and magnitude of the benefit of the device for the individual patient may be variable, considering the performance of the device for the patient's condition. Of note, there are already stool based tests for CRC/APL detections approved by the FDA; the availability of this test, provides patients with another option in the screening for CRC/APL, that may have added value over FIT in the detection of CRC.

The probable risks associated with the use of this device, are mainly due to 1) false positives, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the health care provider. There is minimal probable risk with the collection of stool for the use of this device, since it is noninvasive. When used for screening, a positive result should be followed by colonoscopy for diagnosis. A false positive result could result in an additional invasive screening procedure, such as colonoscopy, and thus unnecessarily expose patients to the attendant risks associated with such a procedure. Rare serious adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to sedation. It is important to note that the specificity of this device also decreases with increasing age. A false negative result with Cologuard Plus™ could potentially delay colonoscopy and delay diagnosis of disease such as colorectal cancer or advanced precancerous lesions. The consequences of false negatives could be quite serious, such as progression of disease, such as CRC to a more advanced, and less treatable stage. One of the concerns around the risks of this device, include the imperfect sensitivity for Stage I CRC (88.0%), Stage II CRC (92.9%) and APL (43.3%). To this end, the patient and provider labeling and instructions for use contains specifications for the performance of this device, including the sensitivity, specificity, PPV and NPV. In addition, there is specific language throughout the labeling, in the Warnings and Precautions sections, including the patient brochure, that says:

- A negative Cologuard Plus test result does not guarantee the absence of cancer or advanced precancerous lesions. Patients with a negative Cologuard Plus™ test result should continue participating in colorectal screening programs at the appropriate guideline recommended levels.

Additional risks include misinterpretation of results of this test by the health care provider. Despite the mitigations of the labeling, there is residual probable risk that the clinician may not fully understand what a positive or negative result from this test means clinically. This risk has been addressed by provided clear summative information on device performance in the Clinician Brochure.

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

Patient Perspective

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

In conclusion, given the available information above, the data support that for the qualitative detection of colorectal advanced neoplasia (CRC/APL) associated with Cologuard Plus methylated DNA markers and the presence of occult hemoglobin in human stool, the probable benefits of Cologuard Plus™ outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the BLUE- C clinical study supports the effectiveness of Cologuard Plus to screen for the presence of CRC or APL in adults of either sex, 45 years or older, who are at average risk for CRC.

XIV. CDRH DECISION

CDRH issued an approval order on October 03, 2024.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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