SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Blood-based Qualitative Colorectal Cancer Screening Test

Device Trade Name: Shield

Device Procode: PHP

Applicant's Name and Address: Guardant Health, Inc
505 Penobscot Dr.
Redwood City, CA 94063

Date(s) of Panel Recommendation: May 23, 2024

Premarket Approval Application (PMA) Number: 230009

Date of FDA Notice of Approval: July 26, 2024

II. INDICATIONS FOR USE

Intended Use:
The Shield test is a qualitative, in vitro diagnostic test intended to detect colorectal cancer derived alterations in cell-free DNA from blood collected in the Guardant Shield Blood Collection Kit.

Shield is intended for colorectal cancer screening in individuals at average risk of the disease, age 45 years or older. Patients with a positive result should be followed by colonoscopy. Shield is not a replacement for diagnostic colonoscopy or for surveillance colonoscopy in high-risk individuals.

This test is performed at Guardant Health, Inc.

III. PRECAUTION

Based on data from clinical studies, Shield has limited detection (55%-65%) of Stage I colorectal cancer and does not detect 87% of precancerous lesions. One out of 10 patients with a negative Shield result may have a precancer that would have been detected by a screening colonoscopy. Shield demonstrated high detection of Stages II, III, and IV colorectal cancer.

IV. CONTRAINDICATIONS

The Shield test is NOT indicated for use for patients that have the following:

- Personal history of colorectal cancer (CRC), adenomas, or other related cancers
- Family history of CRC, defined as having one or more first-degree relative (parent, sibling, or child) diagnosed with CRC at any age
- Positive result on another colorectal cancer screening method within the last six months, or:
    - 12 months for fecal occult blood test (FOBT) or fecal immunochemical test (FIT)
    - 36 months for FIT-DNA test
- Personal history of any of the following high-risk conditions for colorectal cancer:
    - Inflammatory Bowel Disease (IBD), including chronic ulcerative colitis (CUC) and Crohn's disease
    - Familial adenomatous polyposis (FAP)
    - Other hereditary cancer syndromes including but not limited to:
        - Hereditary non-polyposis colorectal cancer syndrome (HNPCC) or "Lynch Syndrome", Peutz- Jeghers Syndrome, MUTYH Polyposis (MAP), Gardner's Syndrome, Turcot's (or Crail's) Syndrome, Cowden's Syndrome, Juvenile Polyposis, Cronkhite-Canada Syndrome, Neurofibromatosis and Familial Hyperplastic Polyposis

V. LIMITATIONS

- Providers should discuss the most appropriate screening test to use with patients depending on their medical history and individual circumstances. The Shield test is not intended as a screening test for individuals who are at high risk for CRC.
- Shield has limited ability to prevent the development of colorectal cancer from advanced precancerous lesions and lower detection rates for Stage I CRC, given the current data available.
    - Shield has lower performance of stage I CRC [54.5% (12/22); 95%CI (34.7%, 73.1%)]. The majority [6/10] of missed Stage I cancers were less than 10mm. Shield did not detect CRC lesions smaller than 10mm [0% (0/6); 95% CI (0.0% - 39.0%)].
    - Shield may fail to detect as many as 88.7% of patients with advanced precancerous lesions which can later become neoplastic because of its limited ability for the detection of advanced adenomas [(13.2% (147/1116); 95%CI (11.3, 15.3)].
    - Shield has a false negative rate of 17% for colorectal cancer, meaning 17 of 100

people who have colorectal cancer will incorrectly have a Shield negative result.

  - Shield has a false positive rate of 10%, meaning one of 10 people who do not have Advanced Neoplasia (colorectal cancer or advanced adenoma) will have a false positive test result.

- CRC screening guideline recommendations vary for persons over the age of 75. The decision to screen patients over the age of 75 should be made on an individualized basis in consultation with a healthcare provider.
- A positive Shield test result suggests patients may have colorectal cancer or advanced adenoma. Patients with a positive result should be followed by colonoscopy.
- A negative Shield test result does not guarantee absence of colorectal cancer or advanced adenoma. Patients with a negative result should continue participating in colorectal cancer screening programs, at the appropriate guideline recommended intervals.
  - One out of 10 patients testing negative will be falsely reassured that they are negative for advanced adenoma, given the negative predictive value for advanced adenoma of 90%.
  - One out of 1000 patients testing negative will be falsely reassured that they are negative for CRC, given the negative predictive value of 99.9%.
- A false positive result may occur when the Shield test generates a positive result while a colonoscopy will not find colorectal cancer or advanced adenoma. A false negative result may occur when the Shield test does not detect a colorectal tumor signal while a colonoscopy identifies a colorectal cancer.
- The performance of Shield has been established in a prospectively designed, cross-sectional study. The benefits and risks of programmatic colorectal screening (i.e., repeated testing over an established period of time) with Shield has not been studied.
- Non-inferiority or superiority of Shield sensitivity as compared to other recommended screening methods for colorectal cancer or advanced adenoma has not been established.
- Cross-reactivity was observed in analytical studies using samples from subjects with non-CRC cancers, including gastric, pancreatic, liver, bladder, breast, lung, prostate, ovarian, melanoma and kidney cancers.
- Consult the Guardant Shield Blood Collection Kit (BCK) instructions for use (LBL-000324), for precautions and limitations specific to the collection and shipping of blood samples.

VI. DEVICE DESCRIPTION

Shield is a next generation sequencing based qualitative test to detect genomic (somatic mutations) and epigenomic alterations (methylation and fragmentation patterns) associated

with colorectal cancer from whole blood samples collected from individuals at average risk for CRC. These samples are shipped to Guardant Health, where cfDNA is extracted from the plasma component of whole blood and prepared for analysis through the DNA sequencing workflow. The resulting cfDNA data are then analyzed using proprietary bioinformatics algorithms trained to detect the presence of colorectal cancer associated signals. Following analysis, a test report is generated for the sample. This test yields a final qualitative test result of "Positive" or "Negative". Patients with a positive result may have colorectal cancer or advanced adenomas and should be followed by colonoscopy.

Reagents, materials, and equipment needed to perform the test are used exclusively in the Guardant Health Clinical Laboratory. The reagents distributed outside of Guardant Health are contained within the blood collection tubes (BCTs) that are part of the Shield Blood Collection Kit (BCK).

The following components are required for the use of Shield:

Shield Blood Collection Kit:

- Guardant cfDNA blood collection tubes
- BCT label with 2D Barcode
- Biohazard specimen bag
- Foam Tray
- Absorbent sheet
- BCK barcode sheet
- BCK Instructions for Use

The Guardant Shield Blood Collection Kit (BCK) comprises all components used in the collection, stabilization, packaging, and transportation of whole blood samples and is the only test component intended for external distribution (i.e., the Shield test itself is performed in Guardant's clinical laboratory). The kit contains four Guardant-labeled blood collection tubes and packaging material with instructions for kit storage, sample collection, and shipping after samples are collected.

Shield Assay:

The following reagents are required to perform the Shield assay and qualified by Guardant Health, Inc. under the Guardant Health Quality System:

- cfDNA extraction beads and buffers
- cfDNA quantitation dye
- DNA purification magnetic beads
- Methylated DNA detection reagents
- Library preparation enzyme mix
- Library enrichment probes
- DNA control - exogenous
- Next generation sequencing kit

Instrumentation:
The following instruments are required to perform the Shield test and qualified by Guardant Health, Inc. under the Guardant Health Quality System:

- Hamilton Microlab STAR
- Tecan SPARK Microplate Multimode Reader
- QIAGEN QIASymphony SP Instrument
- Illumina NovaSeq Sequencing System

Software:
The Shield Test includes software used for sample processing, data analysis, and report generation. The Shield software is comprised of two software subsystems:

- Guardant Assay Platform: The Guardant Assay Platform (GAP) is a software package that supports the execution of assay specific workflows. It provides common infrastructure and functionalities that are assay agnostic. This infrastructure facilitates the execution of the assay by providing the user a graphic interface for managing samples, executing the test, viewing status, receiving notifications, and viewing results.
- Guardant Screening Software: The Guardant Screening Software (GSS) is a software package that implements the Shield screening assay specific workflows and analytics. The GSS runs and is supported by the infrastructure GAP provides. GSS contains all the data analytics, algorithms, assay logic, and business rules for the Shield test.

Additional information about the Shield test is listed in the instructions for use.

Test Procedure:
The Shield test begins with the collection of whole blood in up to 4 Guardant cfDNA blood collection tubes that are provided as part of the Guardant BCK. The patient specimen is then shipped to Guardant Health. Plasma is isolated from whole blood in each tube and then pooled. The cfDNA is extracted from a minimum of 2 mL of plasma for processing through the DNA sequencing workflow. In the cfDNA workflow, cfDNA is prepared in a manner that allows for simultaneous analysis of genomic and epigenomic changes. Epigenomic modifications may be detected as either altered methylation patterns in cfDNA, or as changes in the cfDNA fragment positions. A library is prepared and enriched for informative genomic regions, followed by sequencing of the enriched library. The resulting cfDNA data are analyzed using proprietary bioinformatics algorithms designed to detect the presence of colorectal neoplasm-associated signals.

Whole Blood Collection and Shipping
Peripheral whole blood is collected in Guardant cfDNA BCTs provided with the kit and is then shipped to Guardant Health at ambient temperature. Prior to blood draw, the Guardant BCK may be stored in conditions consistent with its labeling until the expiration date printed on the BCK label. Complete instructions for sample collection and shipping can be found in the BCK Instructions for Use (LBL-000324).

Plasma Isolation and cfDNA Extraction
Upon receipt, whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. Plasma is isolated from the tubes of whole blood via centrifugation. Plasma is divided into primary and retain aliquots with a minimum volume of 2 mL and a maximum volume of 8 mL in each aliquot. cfDNA (cell-free DNA) is extracted from plasma aliquot using the QIAGEN QIAsymphony SP Instrument qualified by Guardant.

Methylation Partitioning
After extraction, cfDNA is separated into methylated and unmethylated partitions based on the overall methylation state of each molecule. The cfDNA is partitioned based on the differential binding affinity of the methylated nucleic acid molecules to a binding agent (i.e., a binding agent that binds to methylated nucleotides).

Library Preparation and Enrichment
The DNA in each partition is tagged with a distinct set of barcodes, which uniquely identifies the partition associated with every molecule. All partitions are then PCR amplified and enriched via hybridization to oligonucleotides representing genomic regions of interest targeting approximately 1Mb of the human genome.

Pooling and Sequencing
Enriched partitions are pooled and tagged with an index uniquely identifying each sample prior to pooling multiple enriched samples into sequencing pools. Sequencing pools are sequenced on the Illumina NovaSeq Sequencing System qualified by Guardant.

Sequencing Data Analysis
Following sequencing, reads are demultiplexed. The methylation partitions associated with every molecule are identified by unique partition labels added during library preparation to enable differentiation of methylated and unmethylated partitions in the analysis step. Only unique molecules which align to genomic regions within the enrichment panels are leveraged in the downstream algorithms.

Calling Algorithm and Result Reporting
The classification of a clinical sample relies upon the multiple biomarkers derived from cfDNA and known to be distinct between normal and cancer-derived tissues. The Shield test interrogates thousands of individual features that characterize three types of cfDNA signals or patterns: epigenetic changes resulting in the aberrant methylation state, epigenetic changes resulting in the aberrant cfDNA molecule fragmentation patterns, and genomic changes resulting in somatic mutations.

These features are used to generate four individual classification scores: Fragmentomics Mixture Score, Methylation LR Score, Methylation MR score and Somatic mutation detection. Individual scores are combined into the “Integrated Score”, and the MR Score is also evaluated independently. The cfDNA MR Score and cfDNA Integrated Scores, are compared to predefined cutoffs to generate positive vs negative results for each cfDNA MR Call and cfDNA Integrated Call. If either of these calls is positive, then the result is positive. A negative call only occurs when both the cfDNA Integrated Call and the cfDNA MR Call are negative.

These results classify samples as either “Positive” or “Negative”.

Quality Control Measures
Shield includes an exogenous DNA control which is designed to contain known features which would result in a positive classification as well as known negative control features that should not be detected on the Shield test. Additionally, a no template negative control (NTC) is run in parallel with patient samples.

The controls are treated as individual samples with processing starting from methylation partition through cfDNA sequencing workflow. Positive classification and absence of negative control regions for the exogenous DNA control and absence of molecules for the NTC are both required quality control measures for reporting valid patient test results.

In addition to assessing the control performance within a batch, the test utilizes multiple per-sample in-process and post-sequencing analytical metrics from clinical sample data that are specific and informative to sample performance.

VII. ALTERNATIVE PRACTICES AND PROCEDURES

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

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

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

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

VIII. MARKETING HISTORY

The Shield test has not been marketed as an IVD in the United States or any foreign country.

IX. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Due to the nature of the noninvasive blood collection process, potential adverse events (AEs) caused by or related to testing with Shield are unlikely. The primary risk associated with the Shield test is a false assay result (i.e., a false positive or a false

negative result). All positive test results should lead to a colonoscopy.
In the instance of a false negative result on Shield, there is a possibility that a case of colorectal cancer or advanced adenoma could go undetected.

X. SUMMARY OF NONCLINICAL STUDIES

Nonclinical studies were conducted at Guardant Health to evaluate the analytical performance of Shield. The studies are described below.

A. Algorithm Development and Cut-Off Determination

The Shield classification models, including assessment of methylation levels, cfDNA fragmentation patterns and cfDNA genomic alterations, were trained on 1,470 known CRC cases representative of all cancer stages and 2,340 cancer-free controls. A second set of samples including 1,050 known CRC cases across all cancer stages and 710 colonoscopy-confirmed controls without AN was used to test the device.

B. Analytical Sensitivity

Limit of Detection (LoD)

The LoD for Shield was expressed in terms of tumor fraction (estimated by somatic mutation frequency, max-MAF), which is the analyte detected by the assay. The LoD was first established as 0.05% max-MAF using cfDNA from 3 clinical CRC samples diluted with pooled normal samples to create independent pools targeting 6 tumor fractions (MAF) levels from 0.01% to 0.5%. All samples were targeted at an input of 600 Non-Singleton Coverage (NSC) which is a challenging input level that minimally exceeds input QC thresholds based on measured NSC variability in cfDNA. Overall LoD was established based on the Shield device positive call rate, and additional analysis was performed to assess positive call rate of the two underlying callers (Integrated and MR positive call rate) at each of the dilution levels. The integrated positive call rate was 100% for dilution levels of 0.10% and above, while the MR positive call rate was 100% for dilution levels of 0.05% and above, resulting in 100% Shield overall positive call rate for dilution levels of 0.05% and above (Table 1).

Table 1. Positive Call Rate of Shield overall call, Integrated call and MR call in the LoD establishing study

Target Max-MAF (%)	Shield Positive Call Rate (%)	Integrated Positive Call Rate (%)	MR Positive Call Rate (%)
0.01	49% (53/108)	4% (4/108)	49% (53/108)
0.02	86% (95/111)	19% (21/111)	86% (95/111)
0.05	100% (117/117)	62% (72/117)	100% (117/117)
0.10	100% (119/119)	95% (113/119)	100% (119/119)
0.30	100% (119/119)	100% (119/119)	100% (119/119)

Target Max-MAF (%)	Shield Positive Call Rate (%)	Integrated Positive Call Rate (%)	MR Positive Call Rate (%)
0.50	100% (115/115)	100% (115/115)	100% (115/115)

The 0.05% max-MAF LoD was confirmed by testing 3 CRC positive sample pools at 3 dilutions levels (0.05%, 0.07%, 1% MAF). A 100% overall positive call rate was observed for dilution levels of 0.05% and above, verifying the detection capability at 0.05% max-MAF.

A supplemental LoD confirmation study was performed using 2 clinical stage I CRC, 1 stage III CRC, a clinical stage unknown CRC and pooled clinical AA samples diluted with pooled self-declared normal cfDNA to the target dilution levels from 0.01% to 0.07%. 100% positive call rate was observed for all samples tested at targeted tumor fraction of 0.05% and above.

Limit of Blank (LoB)

LoB was evaluated with two healthy donor sample pools in 10 different batches using a unique combination of two critical reagent lots. Each sample was tested in greater than 60 replicates each. A total of 198 sample replicates were assessed. The false positive rate (FPR) for the two sample pools was 0%, and 3%, indicating that the FPR is below 5%. Considering both sample pools, the FPR is 1.52%, with a Clopper Pearson Confidence Interval of [0.39%, 4.72%].

C. Precision

Precision was evaluated with a total of 24 clinical samples, including 20 individual clinical samples and 4 clinical sample pools created by spiking CRC patient plasma into plasma from healthy donors. These 24 samples included 3 positive, 5 low positive, 2 high negative, 9 negative, and 5 borderline (close to the test decision boundary) samples. Precision across runs (batches), operators, instruments, reagent lots, and test days was assessed, in addition to concordance between sequencing instruments and batch pooling tolerance for variation in sequencing read depth. Six batches of samples were tested starting on 6 different days using various combinations of reagent lots (3 groups), instrument lines (2 groups), and operators (3 groups) and each sample was tested in 4-36 replicates. The primary analysis was based on the primary sequencing of replicates from all 6 batches. Following primary sequencing, the same libraries were sequenced in the secondary sequencer to assess instrument to instrument precision. Tolerance to pooling of multiple batches within a sequencing flow cell was also assessed. Each of the 6 batches was sequenced on an individual flow cell to compare to pooling with three batches per flow cell. The PPA and NPA for each sample for each level (e.g., primary analysis, instrument to instrument, and pooling tolerance) is shown in Table 2 below.

Of the 24 samples, 16 samples had overall agreement for final Shield call over 92% based on data from the primary sequencing runs. Eight (8) samples had agreement ranging from 58.33% to 91.67% with integrated score and/or MR score close to their predefined cutoffs.

Table 2. Positive call rates for overall Shield call

Sample ID (A)	Sample Disease Status (B)	Sample Category (C)	Level (D)	Number of Replicates (E)	% Called Positive Shield Test Result (F)	% Called Negative Shield Test Result (G)
CRC1	CRC	Positive	Primary analysis	23	100.0	0.0
			Instrument to instrument	23	100.0	0.0
			Pooling tolerance	23	100.0	0.0
CRC2	CRC	Positive	Primary analysis	18	100.0	0.0
			Instrument to instrument	18	100.0	0.0
			Pooling tolerance	18	100.0	0.0
CRC3	pool_CRC_and_ self_declared_ cancer_free	Positive	Primary analysis	23	100.0	0.0
			Instrument to instrument	23	100.0	0.0
			Pooling tolerance	23	100.0	0.0
CRC4	CRC	Low Positive	Primary analysis	12	91.67	8.33
			Instrument to instrument	12	91.67	8.33
			Pooling tolerance	12	91.67	8.33
CRC5	CRC	Low Positive	Primary analysis	12	100.0	0.0
			Instrument to instrument	12	100.0	0.0
			Pooling tolerance	12	100.0	0.0
CRC6	CRC	Low Positive	Primary analysis	9	100.0	0.0
			Instrument to instrument	10	100.0	0.0
			Pooling tolerance	10	100.0	0.0
CRC7	pool_CRC_and_ self_declared_ cancer_free	Low Positive	Primary analysis	28	100.0	0.0
			Instrument to instrument	28	100.0	0.0
			Pooling tolerance	28	100.0	0.0
CRC8	pool_CRC_and_ self_declared_ cancer_free	Low Positive	Primary analysis	29	100.0	0.0
			Instrument to instrument	29	100.0	0.0
			Pooling tolerance	29	100.0	0.0
CRC9	CRC	Borderline	Primary analysis	4	100.0	0.0
			Instrument to instrument	4	100.0	0.0
			Pooling tolerance	4	100.0	0.0
CRC10	CRC	Borderline	Primary analysis	12	58.33	41.67
			Instrument to instrument	12	50.00	50.00
			Pooling tolerance	12	66.67	33.33

Sample ID (A)	Sample Disease Status (B)	Sample Category (C)	Level (D)	Number of Replicates (E)	% Called Positive Shield Test Result (F)	% Called Negative Shield Test Result (G)
CRC11	CRC	Borderline	Primary analysis	17	52.94	47.06
			Instrument to instrument	18	55.56	44.44
			Pooling tolerance	17	76.47	23.53
Healthy 1	self_declared_ cancer_free	Borderline	Primary analysis	17	11.76	88.24
			Instrument to instrument	17	11.76	88.24
			Pooling tolerance	17	23.53	76.47
CRC12	pool_CRC_and_ self_declared_ cancer_free	Borderline	Primary analysis	12	91.67	8.33
			Instrument to instrument	12	91.67	8.33
			Pooling tolerance	12	91.67	8.33
Healthy 2	self_declared_ cancer_free	High Negative	Primary analysis	30	6.67	93.33
			Instrument to instrument	30	3.33	96.67
			Pooling tolerance	30	3.33	96.67
Healthy 3	self_declared_ cancer_free	High Negative	Primary analysis	30	16.67	83.33
			Instrument to instrument	30	13.33	86.67
			Pooling tolerance	30	13.33	86.67
Healthy 4	self_declared_ cancer_free	Negative	Primary analysis	30	3.33	96.67
			Instrument to instrument	30	0.0	100.0
			Pooling tolerance	30	3.33	96.67
Healthy 5	self_declared_ cancer_free	Negative	Primary analysis	30	6.67	93.33
			Instrument to instrument	30	0.0	100.0
			Pooling tolerance	30	6.67	93.33
Healthy 6	self_declared_ cancer_free	Negative	Primary analysis	36	2.78	97.22
			Instrument to instrument	36	0.0	100.0
			Pooling tolerance	36	13.89	86.11
Healthy 7	self_declared_ cancer_free	Negative	Primary analysis	29	13.79	86.21
			Instrument to instrument	29	10.34	89.66
			Pooling tolerance	29	6.90	93.10
Healthy 8	self_declared_ cancer_free	Negative	Primary analysis	29	17.24	82.76
			Instrument to instrument	29	17.24	82.76
			Pooling tolerance	29	13.79	86.21
Healthy 9	self_declared_ cancer_free	Negative	Primary analysis	34	0.0	100.0
			Instrument to instrument	34	0.0	100.0

Sample ID (A)	Sample Disease Status (B)	Sample Category (C)	Level (D)	Number of Replicates (E)	% Called Positive Shield Test Result (F)	% Called Negative Shield Test Result (G)
			Pooling tolerance	34	0.0	100.0
Healthy 10	self_declared_ cancer_free	Negative	Primary analysis	27	0.0	100.0
			Instrument to instrument	27	0.0	100.0
			Pooling tolerance	25	0.0	100.0
Healthy 11	self_declared_ cancer_free	Negative	Primary analysis	29	3.45	96.55
			Instrument to instrument	29	3.45	96.55
			Pooling tolerance	29	0.0	100.0
Healthy 12	self_declared_ cancer_free	Negative	Primary analysis	16	0.0	100.0
			Instrument to instrument	16	0.0	100.0
			Pooling tolerance	16	0.0	100.0

Additional analysis was performed based on data from the primary sequencing runs to assess precision with respect to the underlying Integrated and MR Scores and the corresponding predefined cutoffs and calls. With regards to their scores, samples are classified as positive for integrated call (> -1.73) or negative (<= -1.73); samples are classified as positive for MR call (> -11.06) or negative (<= -11.06). Of the 24 samples, 16 samples had agreement for both integrated call and MR call ranging from 89.66% to 100%. For 5 samples with integrated scores close to the -1.73 cut-off, ranging from -2.0488 to -1.3562, agreement for reproducibility ranged from 52.94% to 58.33%. For 4 samples with MR scores close to the -11.06 cut-off, ranging from -11.5505 to -10.8642, agreement for reproducibility ranged from 50.00% to 88.24%. The observed integrated score and/or MR scores of these 7 samples were close to their respected cut-off, thus explaining the disagreement of Shield overall results near the threshold. These results are presented in Table 3 below.

Table 3. Positive call rates for Integrated call and MR call

Sample ID	Sample Disease Status	Sample Category	Level	Number of Replicates	% Called Positive Int Caller	% Called Negative Int Caller	Mean Int Score	% Called Positive MR Caller	% Called Negative MR Caller	Mean MR Score
CRC 1	CRC	Positive	Primary analysis	23	100.0	0.0	3.2229	100.0	0.0	-7.7724
			Instrument to instrument	23	100.0	0.0	3.1107	100.0	0.0	-7.8003
			Pooling tolerance	23	100.0	0.0	3.0405	100.0	0.0	-7.7967
CRC 2	CRC	Positive	Primary analysis	18	100.0	0.0	1.7009	100.0	0.0	-8.1913
			Instrument to instrument	18	100.0	0.0	1.7691	100.0	0.0	-8.1899
			Pooling tolerance	18	94.44	5.56	1.7554	100.0	0.0	-8.1412

Sample ID	Sample Disease Status	Sample Category	Level	Number of Replicates	% Called Positive Int Caller	% Called Negative Int Caller	Mean Int Score	% Called Positive MR Caller	% Called Negative MR Caller	Mean MR Score
CRC 3	pool_CRC_and_self_declared_cancer_free	Positive	Primary analysis	23	91.3	8.7	0.387	100.0	0.0	-8.7927
			Instrument to instrument	23	95.65	4.35	0.3208	100.0	0.0	-8.7912
			Pooling tolerance	23	95.65	4.35	0.3348	100.0	0.0	-8.7934
CRC 4	CRC	Low Positive	Primary analysis	12	91.67	8.33	0.9091	50.0	50.0	-10.8642
			Instrument to instrument	12	91.67	8.33	0.9321	66.67	33.33	-11.0013
			Pooling tolerance	12	91.67	8.33	0.9798	58.33	41.67	-10.884
CRC 5	CRC	Low Positive	Primary analysis	12	100.0	0.0	0.5933	100.0	0.0	-10.0333
			Instrument to instrument	12	91.67	8.33	0.4539	100.0	0.0	-10.058
			Pooling tolerance	12	91.67	8.33	0.4207	100.0	0.0	-10.0666
CRC 6	CRC	Low Positive	Primary analysis	9	100.0	0.0	0.9889	100.0	0.0	-9.9319
			Instrument to instrument	10	100.0	0.0	1.1593	100.0	0.0	-9.9039
			Pooling tolerance	10	100.0	0.0	1.3446	100.0	0.0	-9.8339
CRC 7	pool_CRC_and_self_declared_cancer_free	Low Positive	Primary analysis	28	42.86	57.14	-1.9915	100.0	0.0	-10.2519
			Instrument to instrument	28	42.86	57.14	-1.899	100.0	0.0	-10.2046
			Pooling tolerance	28	42.86	57.14	-1.9634	100.0	0.0	-10.2226
CRC 8	pool_CRC_and_self_declared_cancer_free	Low Positive	Primary analysis	29	44.83	55.17	-1.6539	100.0	0.0	-9.8775
			Instrument to instrument	29	51.72	48.28	-1.6367	100.0	0.0	-9.8776
			Pooling tolerance	29	48.28	51.72	-1.6773	100.0	0.0	-9.8846
CRC 9	CRC	Borderline	Primary analysis	4	100.0	0.0	4.0064	100.0	0.0	-7.5687
			Instrument to instrument	4	100.0	0.0	4.2094	100.0	0.0	-7.6007
			Pooling tolerance	4	100.0	0.0	4.1896	100.0	0.0	-7.3983
CRC 10	CRC	Borderline	Primary analysis	12	58.33	41.67	-1.3562	16.67	83.33	-11.5284
			Instrument to instrument	12	50.0	50.0	-1.469	8.33	91.67	-11.6253
			Pooling tolerance	12	66.67	33.33	-1.2061	16.67	83.33	-11.3715
CRC 11	CRC	Borderline	Primary analysis	17	52.94	47.06	-1.6064	11.76	88.24	-11.5505
			Instrument to instrument	18	55.56	44.44	-1.6252	11.11	88.89	-11.5562
			Pooling tolerance	17	70.59	29.41	-1.1275	29.41	70.59	-11.2605
Healthy 1	self_declared_cancer_free	Borderline	Primary analysis	17	5.88	94.12	-3.9711	5.88	94.12	-11.536
			Instrument to instrument	17	5.88	94.12	-3.9241	5.88	94.12	-11.5041
			Pooling tolerance	17	5.88	94.12	-3.6675	17.65	82.35	-11.3342

Sample ID	Sample Disease Status	Sample Category	Level	Number of Replicates	% Called Positive Int Caller	% Called Negative Int Caller	Mean Int Score	% Called Positive MR Caller	% Called Negative MR Caller	Mean MR Score
CRC 12	pool_CRC_and_self_declared_cancer_free	Borderline	Primary analysis	12	41.67	58.33	-2.0488	91.67	8.33	-10.384
			Instrument to instrument	12	50.0	50.0	-2.0673	91.67	8.33	-10.3794
			Pooling tolerance	12	41.67	58.33	-2.1271	91.67	8.33	-10.3785
Healthy 2	self_declared_cancer_free	High Negative	Primary analysis	30	3.33	96.67	-3.8289	6.67	93.33	-11.5008
			Instrument to instrument	30	3.33	96.67	-3.7074	3.33	96.67	-11.4957
			Pooling tolerance	30	3.33	96.67	-3.8131	3.33	96.67	-11.503
Healthy 3	self_declared_cancer_free	High Negative	Primary analysis	30	13.33	86.67	-3.1822	3.33	96.67	-11.5721
			Instrument to instrument	30	13.33	86.67	-3.2277	0.0	100.0	-11.6256
			Pooling tolerance	30	13.33	86.67	-3.2514	0.0	100.0	-11.6321
Healthy 4	self_declared_cancer_free	Negative	Primary analysis	30	0.0	100.0	-5.0185	3.33	96.67	-11.6048
			Instrument to instrument	30	0.0	100.0	-5.1673	0.0	100.0	-11.6603
			Pooling tolerance	30	0.0	100.0	-5.0608	3.33	96.67	-11.6075
Healthy 5	self_declared_cancer_free	Negative	Primary analysis	30	0.0	100.0	-4.5761	6.67	93.33	-11.598
			Instrument to instrument	30	0.0	100.0	-4.6926	0.0	100.0	-11.6777
			Pooling tolerance	30	3.33	96.67	-4.5928	6.67	93.33	-11.547
Healthy 6	self_declared_cancer_free	Negative	Primary analysis	36	0.0	100.0	-5.6064	2.78	97.22	-11.6008
			Instrument to instrument	36	0.0	100.0	-5.6882	0.0	100.0	-11.6244
			Pooling tolerance	36	2.78	97.22	-5.5178	13.89	86.11	-11.4241
Healthy 7	self_declared_cancer_free	Negative	Primary analysis	29	10.34	89.66	-3.8179	6.9	93.1	-11.4639
			Instrument to instrument	29	10.34	89.66	-3.9848	3.45	96.55	-11.5322
			Pooling tolerance	29	6.9	93.1	-3.9993	0.0	100.0	-11.538
Healthy 8	self_declared_cancer_free	Negative	Primary analysis	29	3.45	96.55	-4.0325	13.79	86.21	-11.5209
			Instrument to instrument	29	6.9	93.1	-4.0957	10.34	89.66	-11.568
			Pooling tolerance	29	3.45	96.55	-4.0558	10.34	89.66	-11.5503
Healthy 9	self_declared_cancer_free	Negative	Primary analysis	34	0.0	100.0	-4.5917	0.0	100.0	-11.6993
			Instrument to instrument	34	0.0	100.0	-4.6661	0.0	100.0	-11.706
			Pooling tolerance	34	0.0	100.0	-4.7296	0.0	100.0	-11.7092
Healthy 10	self_declared_cancer_free	Negative	Primary analysis	27	0.0	100.0	-4.1272	0.0	100.0	-11.6632
			Instrument to instrument	27	0.0	100.0	-4.1657	0.0	100.0	-11.6713
			Pooling tolerance	25	0.0	100.0	-4.1356	0.0	100.0	-11.6778

Sample ID	Sample Disease Status	Sample Category	Level	Number of Replicates	% Called Positive Int Caller	% Called Negative Int Caller	Mean Int Score	% Called Positive MR Caller	% Called Negative MR Caller	Mean MR Score
Healthy 11	self_ declared_ cancer_free	Negative	Primary analysis	29	3.45	96.55	-4.3717	0.0	100.0	-11.685
			Instrument to instrument	29	3.45	96.55	-4.3397	0.0	100.0	-11.6784
			Pooling tolerance	29	0.0	100.0	-4.3482	0.0	100.0	-11.656
Healthy 12	self_ declared_ cancer_free	Negative	Primary analysis	16	0.0	100.0	-4.7814	0.0	100.0	-11.7901
			Instrument to instrument	16	0.0	100.0	-4.6508	0.0	100.0	-11.7691
			Pooling tolerance	16	0.0	100.0	-4.6448	0.0	100.0	-11.7984

A summary of the variance component analysis for integrated score and MR score evaluated in the precision study is presented in Table 4 and Table 5, respectively. The results are based on data from both the primary sequencing runs and the runs on the second sequencing instrument. The mean, standard deviation (SD), coefficient of variation (%CV), number of replicates (N) were calculated for each test sample. The overall variability (SD) for integrated score ranged from 0.8685 to 2.3246 (Table 4) and the overall variability (SD) for MR score ranged from 0.2135 to 0.6775 (Table 5).

Table 4. Variance component estimates for the Shield test Integrated score for each clinical sample

Clinical Sample ID	Integrated Score		SD	Variance Component Estimates for the Shield Test Integrated Score							N**
	Mean	%CV	Total	Total	Between-Run					Repeatability Within-Batch	
					Between-Lot		Between-Instrument		Between-Operator		
					Sample Prep Reagent	Sequencing Reagent	Sample Prep Instrument	Sequencing			
CRC 9	4.1079	160.35%	6.5872*	43.3911 (100.00%)	35.9180 (82.78%)	0.0000 (0.00%)	6.0286 (13.89%)	1.4444 (3.33%)	0.0000 (0.00%)	0.0000 (0.00%)	8
CRC 1	3.1668	51.64%	1.6352	2.6740 (100.00%)	0.2886 (10.79%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0483 (1.81%)	0.0000 (0.00%)	2.3371 (87.40%)	46
CRC 2	1.735	106.29%	1.844	3.4005 (100.00%)	0.7251 (21.32%)	0.0000 (0.00%)	0.9224 (27.13%)	0.0569 (1.67%)	0.0000 (0.00%)	1.6961 (49.88%)	36
CRC 6	1.0786	132.44%	1.4285	2.0405 (100.00%)	0.5413 (26.53%)	0.4365 (21.39%)	0.0000 (0.00%)	0.0446 (2.18%)	0.0000 (0.00%)	1.0181 (49.89%)	19
CRC 4	0.9206	234.81%	2.1616	4.6724 (100.00%)	0.0000 (0.00%)	0.7598 (16.26%)	0.0000 (0.00%)	0.1272 (2.72%)	0.0000 (0.00%)	3.7854 (81.02%)	24
CRC 5	0.5236	393.07%	2.0581	4.2360 (100.00%)	0.2437 (5.75%)	0.0000 (0.00%)	1.1056 (26.10%)	0.0884 (2.09%)	0.0000 (0.00%)	2.7983 (66.06%)	24
CRC 3	0.3539	632.05%	2.2365	5.0021 (100.00%)	1.2529 (25.05%)	0.0000 (0.00%)	2.3806 (47.59%)	0.0794 (1.59%)	0.3383 (6.76%)	0.9508 (19.01%)	46
CRC 10	-1.4126	-131.49%	1.8575	3.4503 (100.00%)	0.0000 (0.00%)	0.4635 (13.43%)	0.0000 (0.00%)	0.2301 (6.67%)	0.0000 (0.00%)	2.7567 (79.90%)	24

Clinical Sample ID	Integrated Score		SD	Variance Component Estimates for the Shield Test Integrated Score							N**
	Mean	%CV	Total	Total	Between-Run					Repeatability Within-Batch	
					Between-Lot		Between-Instrument		Between-Operator		
					Sample Prep Reagent	Sequencing Reagent	Sample Prep Instrument	Sequencing			
CRC 11	-1.6161	-123.59%	1.9973	3.9890 (100.00%)	0.0000 (0.00%)	2.0345 (51.00%)	0.0000 (0.00%)	0.0839 (2.10%)	0.0000 (0.00%)	1.8706 (46.89%)	35
CRC 8	-1.6453	-102.13%	1.6803	2.8235 (100.00%)	0.0000 (0.00%)	1.0327 (36.58%)	0.0000 (0.00%)	0.0602 (2.13%)	0.0000 (0.00%)	1.7306 (61.29%)	58
CRC 7	-1.9452	-67.76%	1.3182	1.7376 (100.00%)	0.0000 (0.00%)	0.1458 (8.39%)	0.0000 (0.00%)	0.0443 (2.55%)	0.0000 (0.00%)	1.5475 (89.06%)	56
CRC 12	-2.058	-112.95%	2.3246	5.4039 (100.00%)	0.0000 (0.00%)	2.8168 (52.13%)	0.0000 (0.00%)	0.0755 (1.40%)	0.0000 (0.00%)	2.5116 (46.48%)	24
Healthy 3	-3.2049	-58.74%	1.8825	3.5440 (100.00%)	0.6015 (16.97%)	0.0000 (0.00%)	1.2402 (34.99%)	0.0734 (2.07%)	0.3988 (11.25%)	1.2301 (34.71%)	60
Healthy 2	-3.7681	-36.37%	1.3706	1.8785 (100.00%)	0.0000 (0.00%)	0.3691 (19.65%)	0.0000 (0.00%)	0.0639 (3.40%)	0.2089 (11.12%)	1.2366 (65.83%)	60
Healthy 7	-3.9014	-43.64%	1.7028	2.8994 (100.00%)	0.1243 (4.29%)	0.0000 (0.00%)	0.1356 (4.68%)	0.2877 (9.92%)	0.1494 (5.15%)	2.2023 (75.96%)	58
Healthy 1	-3.9476	-38.9%	1.5341	2.3535 (100.00%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0443 (1.88%)	0.4678 (19.88%)	1.8414 (78.24%)	34
Healthy 8	-4.0641	-32.6%	1.326	1.7582 (100.00%)	0.0403 (2.29%)	0.1859 (10.57%)	0.0000 (0.00%)	0.0991 (5.63%)	0.0000 (0.00%)	1.4330 (81.50%)	58
Healthy 10	-4.1465	-28.9%	1.1978	1.4347 (100.00%)	0.0742 (5.17%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0622 (4.34%)	0.0000 (0.00%)	1.2984 (90.49%)	54
Healthy 11	-4.3557	-31.35%	1.3656	1.8650 (100.00%)	0.1918 (10.28%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0258 (1.38%)	0.2349 (12.60%)	1.4125 (75.74%)	58
Healthy 9	-4.6289	-27.66%	1.2801	1.6387 (100.00%)	0.0415 (2.53%)	0.0214 (1.31%)	0.0000 (0.00%)	0.0428 (2.61%)	0.0000 (0.00%)	1.5330 (93.55%)	68
Healthy 5	-4.6344	-34.74%	1.6102	2.5927 (100.00%)	0.3098 (11.95 %)	0.0000 (0.00%)	0.5583 (21.53%)	0.1439 (5.55%)	0.1590 (6.13%)	1.4219 (54.84%)	60
Healthy 12	-4.7161	-18.42%	0.8685	0.7544 (100.00%)	0.0000 (0.00%)	0.1361 (18.04%)	0.0000 (0.00%)	0.0385 (5.10%)	0.3098 (41.06%)	0.2700 (35.79%)	32
Healthy 4	-5.0929	-20.92%	1.0653	1.1348 (100.00%)	0.0000 (0.00%)	0.1472 (12.97%)	0.0000 (0.00%)	0.1414 (12.46%)	0.0000 (0.00%)	0.8462 (74.56%)	60
Healthy 6	-5.6473	-17.10%	0.9656	0.9323 (100.00%)	0.0017 (0.18%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0646 (6.93%)	0.0329 (3.52%)	0.8331 (89.36%)	72

* Results for clinical sample CRC 9 were determined to be numerically unstable and are not recommended for use

** N = Total number of replicates

Table 5. Variance component estimates for the Shield test MR score for each clinical sample

Clinical Sample ID	MR Score		SD	Variance Component Estimates for the Shield Test MR Score							N**
					Between-Run					Repeatability Within-Batch	
					Between-Lot		Between-Instrument		Between-Operator		
	Mean	%CV	Total	Total	Sample Prep Reagent	Sequencing Reagent	Sample Prep Instrument	Sequencing			
CRC 9	-7.5847	-10.94%	0.8298	0.6886 (100.00%)	0.5012 (72.80%)	0.0000 (0.00%)	0.1285 (18.67%)	0.0588 (8.54%)	0.0000 (0.00%)	0.0000 (0.00%)	8
CRC 1	-7.7863	-5.80%	0.4515	0.2038 (100.00%)	0.0415 (20.36%)	0.0002 (0.10%)	0.0214 (10.51%)	0.0053 (2.61%)	0.0000 (0.00%)	0.1354 (66.43%)	46
CRC 2	-8.1906	-8.27%	0.6775	0.4590 (100.00%)	0.1467 (31.97%)	0.0000 (0.00%)	0.1107 (24.12%)	0.0099 (2.16%)	0.0000 (0.00%)	0.1916 (41.75%)	36
CRC 3	-8.7919	-4.25%	0.3735	0.1395 (100.00%)	0.0120 (8.59%)	0.0000 (0.00%)	0.0187 (13.39%)	0.0025 (1.76%)	0.0000 (0.00%)	0.1064 (76.26%)	46
CRC 8	-9.8776	-5.28%	0.5216	0.2721 (100.00%)	0.0950 (34.91%)	0.0000 (0.00%)	0.0264 (9.69%)	0.0021 (0.77%)	0.0131 (4.81%)	0.1355 (49.82%)	58
CRC 6	-9.9171	-4.05%	0.402	0.1616 (100.00%)	0.0678 (41.94%)	0.0554 (34.26%)	0.0164 (10.13%)	0.0051 (3.18%)	0.0000 (0.00%)	0.0169 (10.49%)	19
CRC 5	-10.0456	-5.13%	0.5151	0.2653 (100.00%)	0.0000 (0.00%)	0.1279 (48.20%)	0.0000 (0.00%)	0.0045 (1.68%)	0.0000 (0.00%)	0.1329 (50.11%)	24
CRC 7	-10.2283	-3.01%	0.3083	0.0950 (100.00%)	0.0078 (8.22%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0070 (7.37%)	0.0110 (11.59%)	0.0692 (72.82%)	56
CRC 12	-10.3817	-5.92%	0.6149	0.3781 (100.00%)	0.0000 (0.00%)	0.2385 (63.09%)	0.0000 (0.00%)	0.0018 (0.49%)	0.0000 (0.00%)	0.1377 (36.43%)	24
CRC 4	-10.9327	-3.71%	0.406	0.1648 (100.00%)	0.0000 (0.00%)	0.0952 (57.77%)	0.0082 (4.99%)	0.0551 (33.41%)	0.0000 (0.00%)	0.0063 (3.84%)	24
Healthy 7	-11.498	-3.67%	0.4221	0.1782 (100.00%)	0.0000 (0.00%)	0.0694 (38.94%)	0.0000 (0.00%)	0.0452 (25.35%)	0.0000 (0.00%)	0.0636 (35.71%)	58
Healthy 2	-11.4982	-2.33%	0.2674	0.0715 (100.00%)	0.0019 (2.59%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0016 (2.20%)	0.0205 (28.72%)	0.0475 (66.49%)	60
Healthy 1	-11.52	-3.03%	0.3486	0.1215 (100.00%)	0.0000 (0.00%)	0.0169 (13.93%)	0.0000 (0.00%)	0.0042 (3.44%)	0.0000 (0.00%)	0.1004 (82.63%)	34
Healthy 8	-11.5445	-3.23%	0.3729	0.1390 (100.00%)	0.0125 (8.99%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0256 (18.40%)	0.0202 (14.55%)	0.0807 (58.07%)	58
CRC 11	-11.5534	-3.97%	0.4591	0.2108 (100.00%)	0.0000 (0.00%)	0.0624 (29.59%)	0.0147 (6.99%)	0.0153 (7.26%)	0.0279 (13.22%)	0.0905 (42.93%)	35
CRC 10	-11.5769	-5.50%	0.6367	0.4054 (100.00%)	0.0000 (0.00%)	0.0967 (23.87%)	0.0000 (0.00%)	0.0676 (16.67%)	0.0000 (0.00%)	0.2410 (59.46%)	24
Healthy 3	-11.5988	-2.56%	0.2967	0.0880 (100.00%)	0.0059 (6.72%)	0.0003 (0.33%)	0.0203 (23.10%)	0.0142 (16.10%)	0.0000 (0.00%)	0.0473 (53.74%)	60
Healthy 6	-11.6126	-1.8%	0.2135	0.0456 (100.00%)	0.0000 (0.00%)	0.0032 (7.10%)	0.0000 (0.00%)	0.0093 (20.37%)	0.0000 (0.00%)	0.0331 (72.53%)	72
Healthy 4	-11.6325	-2.1%	0.2426	0.0589 (100.00%)	0.0014 (2.42%)	0.0000 (0.00%)	0.0000 (0.00%)	0.0327 (55.51%)	0.0033 (5.68%)	0.0214 (36.40%)	60

Clinical Sample ID	MR Score		SD	Variance Component Estimates for the Shield Test MR Score							N**
					Between-Run					Repeatability Within-Batch	
					Between-Lot		Between-Instrument		Between-Operator		
	Mean	%CV	Total	Total	Sample Prep Reagent	Sequencing Reagent	Sample Prep Instrument	Sequencing			
Healthy 5	-11.6379	-2.3%	0.2728	0.0744 (100.00%)	0.0067 (9.02%)	0.0000 (0.00%)	0.0067 (9.01%)	0.0300 (40.24%)	0.0000 (0.00%)	0.0311 (41.72%)	60
Healthy 10	-11.6672	-2.46%	0.2866	0.0822 (100.00%)	0.0240 (29.25%)	0.0000 (0.00%)	0.0039 (4.73%)	0.0027 (3.33%)	0.0099 (12.04%)	0.0416 (50.65%)	54
Healthy 11	-11.6817	-2.92%	0.3407	0.1161 (100.00%)	0.0000 (0.00%)	0.0512 (44.13%)	0.0000 (0.00%)	0.0011 (0.95%)	0.0014 (1.20%)	0.0624 (53.73%)	58
Healthy 9	-11.7026	-1.97%	0.2309	0.0533 (100.00%)	0.0024 (4.54%)	0.0000 (0.00%)	0.0054 (10.21%)	0.0015 (2.85%)	0.0000 (0.00%)	0.0439 (82.40%)	68
Healthy 12	-11.7796	-2.80%	0.3294	0.1085 (100.00%)	0.0107 (9.82%)	0.0000 (0.00%)	0.0201 (18.52%)	0.0022 (2.04%)	0.0000 (0.00%)	0.0755 (69.63%)	32

* Results for clinical sample CRC 9 were determined to be numerically unstable and are not recommended for use
** N = Total number of replicates

Based on data from precision and other analytical studies where multiple replicates of the same sample were tested, samples with integrated scores ranging from -2.3590 to -0.1243 yielded less than 95% concordant integrated calls across replicates; samples with MR scores ranging from -11.1935 to -10.6569 yielded less than 95% concordant MR calls across replicates. Based on the clinical study, ~15% samples generated an integrated score within the range that will yield less than 95% concordant call (Table 6), and ~15% samples generated a MR score within the range that will yield less than 95% concordant call (Table 7).

Table 6. Prevalence, agreement rates, and misclassification rates for samples with Shield test Integrated scores close to the decision threshold

Shield Test Integrated Score									
Range of Shield Test Integrated Score	Source of Intermediate Precision Estimate		Distance to Cutoff (SD)	Number of CV Samples Within this Range / Total Samples	Percentage of CV Samples Within this Range	NPA [95% CI]	False Positive Calls / Total Expected Negative Replicates in AV	PPA [95% CI]	False Negative Calls / Total Expected Positive Replicates in AV
	VCA Category	Intermediate Precision Estimate							
[-4.2353, -2.9844]	High Negative	1.2509	-2 to -1	3448 / 7861	43.86%	95.62% [93.99, 96.91]	36 / 822	Not Available	Not Available
[-2.9844, -2.3590]			-1 to -0.5	1217 / 7861	15.48%	90.62% [82.95, 95.62]	9 / 96	22.45% [11.77, 36.62]	38 / 49
[-2.3590, -2.0462]			-0.5 to -0.25	370 / 7861	4.71%	68.75% [41.34, 88.98]	5 / 16	40.48% [29.90, 51.75]	50 / 84
[-2.0462, -1.7335]			-0.25 to Threshold	287 / 7861	3.65%	62.50% [35.43, 84.80]	6 / 16	43.08% [34.43, 52.05]	74 / 130
[-1.7335, -1.3312]	Low Positive	1.6092	Threshold to 0.25	229 / 7861	2.91%	Not Available	Not Available	52.63% [46.20, 59.00]	117 / 247
[-1.3312, -0.9289]			0.25 to 0.5	127 / 7861	1.60%	Not Available	Not Available	68.46% [60.35, 75.82]	47 / 149
[-0.9289, -0.1243]			0.5 to 1	103 / 7861	1.31%	0.00% [0.00, 45.93]	6 / 6	80.00% [70.54, 87.51]	19 / 95
[-0.1243, 1.4849]			1 to 2	59 / 7861	0.75%	Not Available	Not Available	94.58% [91.75, 96.66]	20 / 369

Table 7. Prevalence, agreement rates, and misclassification rates for samples with Shield test MR Scores close to the decision threshold

Shield Test MR Score									
Range of Shield Test MR Score	Source of Intermediate Precision Estimate		Distance to Cutoff (SD)	Number of CV Samples Within this Range / Total Samples	Percentage of CV Samples Within this Range	NPA [95% CI]	False Positive Calls / Total Expected Negative Replicates AV	PPA [95% CI]	False Negative Calls / Total Expected Positive Replicates in AV
	VCA Category	Intermediate Precision Estimate							
[-11.5896, -11.3255]	High Negative	0.2641	-2 to -1	3567 / 7861	45.38%	95.24% [93.50, 96.63]	37 / 777	13.43% [6.33, 23.97]	58 / 67
[-11.3255, -11.1935]			-1 to -0.5	938 / 7861	11.93%	100.00%[90.26, 100.00]	0 / 36	33.33% [17.29, 52.81]	20 / 30
[-11.1935, -11.1274]			-0.5 to -0.25	281 / 7861	3.57%	66.67% [34.89, 90.08]	4 / 12	50.00% [18.71, 81.29]	5 / 10
[-11.1274, -11.0614]			-0.25 to Threshold	209 / 7861	2.66%	66.67% [22.28, 95.67]	2 / 6	Not Available	Not Available
[-11.0614, -10.9603]	CRC Donor*	0.4045	Threshold to 0.25	183 / 7861	2.32%	0.00% [0.00, 45.93]	6 / 6	52.17% [30.59, 73.18]	11 / 23
[-10.9603, -10.8592]			0.25 to 0.5	107 / 7861	1.36%	0.00% [0.00, 45.93]	6 / 6	61.90% [45.64, 76.43]	16 / 42
[-10.8592, -10.6569]			0.5 to 1	99 / 7861	1.26%	Not Available	Not Available	80.00% [51.91, 95.67]	3 / 15
[-10.6569, -0.2524]			1 to 2	84 / 7861	1.07%	0.00% [0.00, 45.93]	6 / 6	91.67% [84.24, 96.33]	8 / 96

*CRC Donor category used in place of Low Positive for Shield Test MR Scores because the Low Positive category only had 1 sample (with 12 reps in the primary analysis). By way of contrast, the Low Positive category for Shield Test Integrated Scores contained data for 65 replicates in the primary analysis derived from 4 unique clinical CRC samples.

D. Analytical Specificity

1) In-silico Analysis of Primers and Probes

The specificity of the primers and probes used in the Shield test was evaluated by testing the potential of the probes and primers to amplify the non-specific products from human DNA and assessing the potential for incorrect results due to commensal microorganism using publicly available in-silico analysis.

The probe specificity was determined by mapping probe sequences to the human genome and performing in silico analyses to detect possible contaminant non-human sequences. Study results found no potential for off-target amplification or binding from human, enterobacterial or viral non-specific targets (common bacterial, fungal, and viral sources that are commonly found in the human blood or on the skin).

2) Cross-Reactivity with Non-Colorectal Cancers and Diseases

Non-CRC Cancers

The potential for cross-reactivity with non-colorectal cancers was evaluated two ways:

1. The incidence of cancer in subjects enrolled in the clinical study were evaluated for a diagnosis of cancer (ECLIPSE) within one year of enrollment (as of March 2024). The rate of non-CRC cancers was compared between participants who had false positive results for advanced neoplasia (AN), and true negative for AN. No statistically significant difference in the incidence rate was observed (0.8% [5/640] versus 0.9% [51/5,502] respectively, adjusted p-value=0.4584).

2. Evaluation of Shield positivity on 218 specimens from subjects with a known diagnosis of a non-CRC cancer. The Shield positivity rates range from 50.0% to 92.9% across 9 cancer types. The samples in the cross-reactivity study were collected from individuals with a known diagnosis of cancer, which is not representative of an asymptomatic intended use population. The result is an overestimation of the non-CRC cancer detection. Table 8 estimates the worst-case scenario for false positives based on the estimated incidence rate and false positive findings. The test is not intended for detection of other cancers.

Table 8. Cross reactivity in the context of Cancer Incident Rates

Cancer Type	Incidence in US in 2022[4]	Incident Rate per 10,000 individuals	Number of positive calls in 10,000 subjects
Bladder	81,180	2.44	2.07
Breast	290,560	8.74	6.99
Gastric*	47,020	1.41	1.30
Kidney	79,000	2.34	1.17
Liver	41,260	1.24	1.09
Lung	236,740	7.12	5.44
Melanoma	99,780	3.00	1.71
Ovarian	19,880	0.60	0.37
Pancreatic	62,210	1.87	1.73
Prostate	268,490	8.07	5.10
Total			26.97
*Gastric cancer incidences were estimated by totaling Esophagus and Stomach cancer incidences			

Non-Cancer Diseases

The potential for non-cancer disease cross-reactivity was evaluated in 2440 subjects from the interim analysis dataset in the ECLIPSE study that did not have CRC or AA detected by colonoscopy. False positive results were evaluated in combination with the disease prevalence which was observed in interim analysis dataset in ECLIPSE to assess the impact of disease on Shield specificity. The results are shown in Table 9 below.

Table 9. Positivity rates for observed co-morbidities within ECLIPSE interim analysis data set

Disease	Number of samples	Prevalence Observed in Interim Analysis Set	Positivity Rate	Projected number of positive Shield calls in10,000 subjects
Hypertension	569	23.3%	10.5%	245.58
Dyslipidemia	515	21.1%	12.4%	262.27
Diabetes Mellitus	266	10.9%	13.9%	151.62
Gastroesophageal Reflux Disease	212	8.7%	8.5%	73.81
Allergy	142	5.8%	3.5%	20.49
Hypothyroidism	140	5.7%	12.9%	73.84
Anxiety	86	3.5%	5.8%	20.48
Depression	85	3.5%	11.8%	41.00
Osteoporosis	76	3.1%	17.1%	53.29
Arthritis	73	3.0%	8.2%	24.59
Asthma	72	3.0%	5.6%	16.69
Constipation	66	2.7%	24.2%	65.57
Benign Prostatic Hypertrophy	64	2.6%	15.6%	40.97
Indigestion	59	2.4%	13.6%	33.02
Drug Allergy	57	2.3%	12.3%	28.80

3) Interfering Substances

To evaluate the impact of potential endogenous interfering substances (unconjugated bilirubin, conjugated bilirubin, triglycerides, gDNA, albumin, and hemoglobin) on the performance of Shield, four clinical specimens (2 minimally manipulated positive and 2

negative samples) were tested in 8 replicates in the presence and absence of the above-mentioned substances at levels shown in Table 10 below. No interference with the Shield test was observed for any of the substances at the concentrations tested.

Table 10. Endogenous Interfering Substances Tested

Interferent	Amount added [g/L or as specified]
Albumin	60
Conjugated Bilirubin	0.4
Unconjugated Bilirubin	0.4
Hemoglobin	10
Triglycerides, Total	15
gDNA	100 ng per replicate

E. Cross-Contamination / Carry Over

Cross-contamination and carry over was evaluated using a checkerboard design alternating pre-characterized high positive male donor samples and low negative female donor samples in two plates processed consecutively using the same line of instruments. A total of 47 samples of each type were tested in 2 batches for a total of 188 replicates. The PPA and NPA for first plate is 100% and the PPA and NPA for the second plate is 100.00% and 97.83%, respectively. One false positive Shield test result was observed across the 94 Low Negative sample replicates. The false positive was from a single replicate among 31 replicates tested of a Low Negative sample, due to a false positive Integrated Call. The Integrated Score for that replicate was 2.414 x SD (per Precision Study) from the mean of the 31 replicates for the discordant Low Negative sample. The probability of observing a maximum deviation from the mean 2.5 x SD or greater across a set of 31 normally distributed scores is approximately 17.6%, supporting the hypothesis that the outlying value is not necessarily attributed to a mechanism outside of expected variability in Shield Test Integrated Scores.

F. Robustness

Assay Workflow Guardbanding

The objective of the robustness study was to evaluate the tolerance of the Shield Test to variation in critical assay workflow parameters in the categories listed below:

- Reagent Volumes relative to instrument tolerances for key reagents
- Incubation Times including library enrichment and cfDNA hybridization times
- Hold Point durations for extracted cfDNA and samples during processing

- Temperature variations during the enrichment hybridization process.

Six (6) positive and seven (7) negative clinical specimens including samples that consisted of extracted cfDNA from pooled self-declared healthy individuals spiked with cfDNA from clinical CRC positive samples to varying levels of the Shield component test scores were tested in 8-15 replicates. A minimum of 6 positive and 6 negative samples were tested per guardbanded condition and compared to baseline reference samples. PPA for the overall Shield call observed at all conditions tested is 100%, and NPA is 100% except three conditions. The variation of scores between the control and testing conditions is consistent with the assay measurement variability observed in the precision study, indicating that the Shield performance is robust with respect to critical assay workflow parameters.

Input Guardbanding

An input guardbanding study was conducted to evaluate the robustness of the Shield assay at different input levels. cfDNA workflow for Shield Test uses post-sequencing metrics to evaluate if a sufficient amount of input material was sequenced successfully and one of post-sequencing metrics reflective of DNA molecule recovery is Non-Singleton Coverage (NSC), which is highly correlated with the input amount into the assay.

The robustness of Shield to the cfDNA input level was assessed with 5 cfDNA positive and 4 cfDNA negative samples including samples with scores near the clinical decision cutoff that were selected at NSC > 1000 and diluted to NSC ranging from 400 (below the QC threshold of 500) to 1000. Each of the 5 positive and 4 negative samples was tested in 5 replicates at NSC level of 400, 600, 800 and 1000, while 1 replicate was tested for 3 positive and 3 negative samples at high cfDNA input (2000-5000 NSC), yielding a total of 186 replicates. The study results demonstrated at least 95% agreement for positive and negative samples, indicating the robustness of the assay at low cfDNA input levels.

G. Stability

Whole Blood Stability

Whole blood stability was assessed using blood samples collected from 70 self-declared cancer-free donors and 60 independent pooled clinical positive samples at different time point (day 1, day 6, day 8/9 and day 10) to support stability of whole blood specimens collected in BCTs for up to 9 days across the expected range of sample transport and storage conditions. An additional stability study was also performed to demonstrate that the device performance for whole blood processed at Day 1 can represent the device performance for samples processed on the date of collection. Plasma was isolated from two BCTs per donor at each of the isolation time points and resulting plasma was processed through the downstream Shield workflow steps. A replicate for each sample was processed for this study, and classification calls were compared between reference and storage conditions.

PPA is 100% for both winter and summer shipping conditions, while NPA for summer shipping condition are 96.15%, 96.97% and 96.30% at day 6, day 8, and day 10, respectively and NPA for winter shipping condition at day 6 is 93.10%. No significant

difference in Shield integrated score and MR score was observed at different timepoint for each sample transport and storage condition tested.

Plasma Stability: Short-term

A short-term plasma stability study was conducted to evaluate the stability of plasma collected in BCT tubes for short-term storage conditions in the Shield workflow. A total of 29 healthy and 42 CRC samples were tested at 3 time-points (day8, day 16 and day 31 with 12-15 CRC samples per timepoint) and 2-3 freeze-thaw cycles. Each healthy donor plasma sample was tested in 2-3 replicates at each timepoint (3 replicates at baseline and 2 replicates at later timepoints) and 1 replicate was tested per CRC donor sample per timepoint. PPAs for overall Shield call of CRC donor samples were 100%, while 7 samples from self-declared cancer free donors yielded discordant calls. Based on analysis of the integrated score and MR score, the difference between time points and freeze-thaw cycles is within the variation of scores observed in the precision study and discordant calls are due to samples with integrated score and MR score close to the cutoff. The results support the short-term stability of plasma isolated from BCTs for 30 days with up to 3 freeze-thaw cycles when stored at -80°C.

Plasma Stability: Long-term

A long-term plasma stability study was designed to evaluate the impact of long-term plasma storage and demonstrate equivalence to plasma processed within 30 days of isolation from BCT tubes. A total of 30 healthy and 70 CRC samples are to be tested across 4 time-points. This study is ongoing and results were obtained for the T0 and T1 (7 month) time-points. No statistically significant degradation in stability was observed at 7 months. Currently, this study has demonstrated stability for at least 6 months.

cfDNA stability

cfDNA stability was evaluated with 4 clinical samples and 2 minimally manipulated cfDNA samples constructed by mixing cfDNA extracted from normal plasma pool and cfDNA extracted from CRC plasma to varying levels of the Shield component test scores. Samples were tested in 20 replicates at each timepoint (e.g., baseline, 4 months, 7 months, 10 months and 13 months). No significant differences in either integrated score and MR score were observed between baseline and later timepoint, demonstrating cfDNA stability for 12 months when stored at -20°C.

Reagent Shelf-Life Stability

Shelf-life stability testing for the Shield reagents was conducted by evaluating the end-to-end functional performance of 3 lots of reagents over a period of 13 months to support a 12 month shelf-life claim. Each reagent lot comprised unique lots of the individual reagents, which were tested at various time points over 13 months. Reagents stored at ≤ -20°C were freeze-thawed 3 times prior to testing. A set of cfDNA samples consisting of extracted cfDNA from pooled self-declared healthy individuals spiked with cfDNA from clinical CRC positive samples to varying levels of the Shield component test scores were evaluated in 20 replicated at each time point (e.g., baseline, 4 months, 7 months, 10 months and 13 months). The results of the reagent shelf-life stability study showed that there was no change in assay performance throughout the 13 months of testing for all reagents

demonstrating that Shield assay reagents are stable for 12 months.

On-Board Stability
The purpose of the on-board stability studies was to determine reagent stability after using and holding reagents under different process steps. All reagents required for the Shield workflow were tested. Three clinical and minimally manipulated positive samples and three clinical negative samples were tested in 16-24 replicates per condition to evaluate the tolerance of the Shield assay to the final onboard hold conditions tested. No significant difference in assay performance or Shield scores between test condition and reference condition were observed. Samples were processed with the Shield workflow with the following hold points and were shown to be stable for the stated hold times:

- Shield reagents are stable for at least 30 minutes at room temperature on the Hamilton Microlab STAR deck.
- Samples are stable at 2-8°C in PCR master mix for at least 24 hours.

In-Use Stability
An in-use stability study was conducted to evaluate the stability of 3 hold-points in the Shield workflow. The in-use stability study utilized 1,116 sample replicates representative of the positive and negative Shield component scores to verify the following hold conditions:

- Samples stored after Library Prep Clean-up at -15 to -25°C for 20 days with one freeze-thaw cycle
- Samples stored after Enrichment Transfer at -15 to -25°C for 14 days with one freeze-thaw cycle
- Samples stored after Sequencing Normalization at -15 to -25°C for 13 days with one freeze-thaw cycle

Test condition samples were tested for the listed conditions and compared against the reference condition samples that were not held during processing. There was no significant difference between the test or reference samples for the 3 tested hold conditions, supporting the use of the hold points as part of the assay process.

Animal Studies

N/A

Additional Studies

N/A

XI. SUMMARY OF PRIMARY CLINICAL STUDY

The pivotal study ECLIPSE ("Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode") was conducted to generate data to support the safety and effectiveness of Shield as a blood-based screening test for the detection of alterations

associated with the presence of colorectal cancer (CRC) from whole blood samples. To evaluate the performance of Shield, the test result (negative or positive) was compared with the histopathological result from colonoscopy examination and histopathological diagnosis of all lesions discovered during the colonoscopy. Based on this comparison, Shield sensitivity (true positive fraction) was 83.1% (54/65) for subjects with a histopathological diagnosis of CRC and 13.2% (147/1116) for subjects with a diagnosis of advanced adenoma (Category 2a - 2e, Table 6). For subjects without a diagnosis of CRC or AA, Shield specificity (true negative fraction) was 89.6% (5982/6680).

A. Study Design

The ECLIPSE study was a multi-site, prospective, non-randomized, observational study designed to evaluate the clinical performance of Shield in patients 45 – 84 years of age who were of average risk for CRC. Patients eligible for CRC screening and intending to undergo colonoscopy were enrolled in the study. The study enrolled a total of 24,876 subjects from 265 sites across the US between October 8, 2019, and the data cutoff on September 30, 2022. Blood samples were collected from all patients who consented to enroll in the study and met eligibility criteria. Blood collection was performed prospectively using Guardant Blood Collection Kits from all enrolled subjects prior to the patient undergoing standard of care colonoscopy and were processed and analyzed at Guardant Health. Performance of the Shield test was compared against colonoscopy result. Central pathology reviews were conducted for lesion classification. The lesion of greatest clinical significance was used to classify each subject into one of the histopathology categories according to the pre-specified standards outlined in Table 11. These categories were used to designate the reference result for the purpose of determining test sensitivity and specificity, positive predictive value and negative predictive value.

Table 11. Colonoscopy/Histopathology Diagnosis Category Descriptions

Category	Colonoscopy Findings	Class for Reference Result
1	Colorectal cancer, any stage	**CRC**
2	Advanced adenoma	**AA**
2a	Carcinoma in situ, any size	
2b	High-grade dysplasia, any size	
2c	Villous growth % (>25%), any size	
2d	Tubular adenoma, ≥10 mm	
2e	Serrated lesion, ≥10 mm (includes sessile serrated adenoma/polyp)	
3	Non-advanced adenoma, >3 adenomas, <10 mm	**Non-AN**
4	Non-advanced adenoma, 1 or 2 adenomas, >5 mm, <10 mm	
5	Non-advanced adenoma, 1 or 2 adenomas, ≤5 mm	
6	Negative, or other findings	
7	Not evaluable	

B. Clinical Inclusion and Exclusion Criteria

Enrollment in the ECLIPSE study was limited to subjects who met the following inclusion criteria:

- Aged 45 to 84 years at time of consent.
- Intended to undergo screening colonoscopy.
- Considered by a physician or healthcare provider as being of average risk for CRC.
- Willing to consent to blood draw pre-bowel preparation administration prior to undergoing colonoscopy within 60 days (amended to 6 months) of the date of the investigational blood draw.
- Willing to consent to follow-up for two years as per protocol.

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

- Undergoing colonoscopy for investigation of symptoms.
- Has undergone colonoscopy within preceding 9 years.
- Positive FIT/fecal occult blood test result within the previous 6 months.
- Has completed Cologuard or Epi proColon testing within the previous 3 years.
- Personal history of CRC.
- Personal history of any malignancy (patients who have undergone surgical removal of skin squamous cell cancer may be enrolled provided the procedure was completed at least 12 months prior to the date of provision of informed consent for the study).
- Known diagnosis of inflammatory bowel disease.
- Currently taking any anti-neoplastic or disease-modifying anti-rheumatic drugs.
- Family history of CRC, defined as having one or more first-degree relatives (parent, sibling, or child) with CRC at any age.
- Known hereditary/germline risk of CRC (for example, Lynch syndrome or hereditary nonpolyposis CRC, or familial adenomatous polyposis).
- Any major physical trauma (e.g., disruption of tissue, surgery, organ transplant, blood product transfusion) within the 30 days leading up to the provision of informed consent.
- Known medical condition which, in the opinion of the Investigator, should preclude enrollment into the study.
- Participation in a clinical research study in which an experimental medication has been administered or may be administered within the 30 days leading up to providing informed consent or may be administered through the time of colonoscopy.

C. Follow-up Schedule

All patients were contacted at 1 and 2 years after blood sample collection to confirm diagnoses of interval malignancies.

D. Clinical Performance Measures

Primary objectives

The primary objective of this study was to establish the performance characteristics of

the Shield test sensitivity for CRC (category 1, Table 11) and specificity of non-advanced neoplasia (categories 3, 4, 5, and 6), Table 11) in average-risk patients against the clinical results defined by colonoscopy/histopathology diagnosis. The primary performance measures were analyzed based on the Guardant's predefined acceptance criteria for sensitivity of CRC and specificity for AN:

- With regards to the Shield sensitivity for subjects with CRC, the lower bound of the two-sided 95% confidence interval must be greater than 65%.
- With regards to the Shield specificity for subjects with non-AN, the lower bound of the two-sided 95% confidence interval must be greater than 85%.

Secondary objectives

The secondary objective was to establish the sensitivity of the Shield test in the detection of AA in average-risk patients. No performance goal was predefined for the secondary performance measure of AA sensitivity.

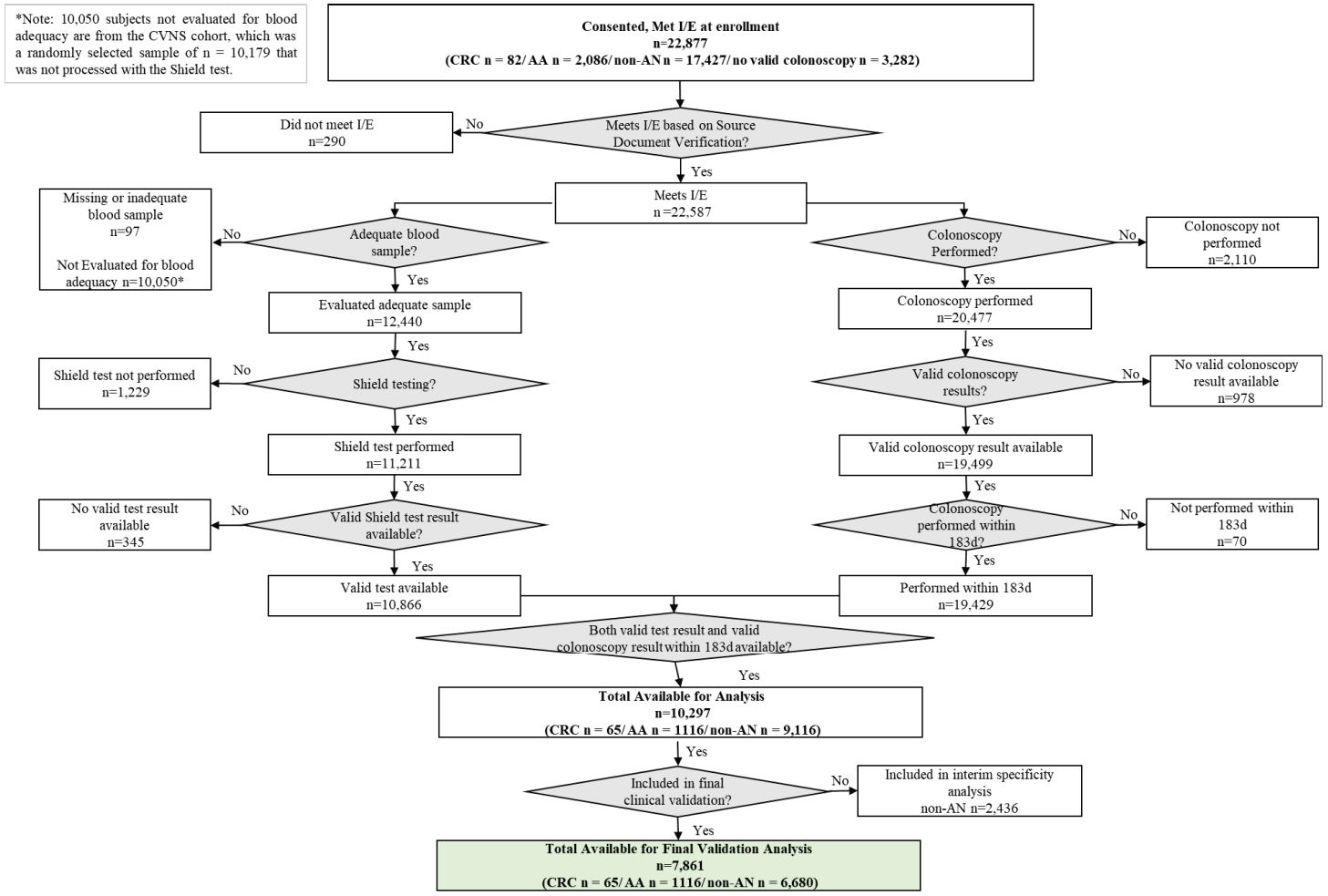
E. Accountability of PMA Clinical Validation Dataset

Samples were collected from a total of 24,876 subjects at 265 sites for the Shield test. The disposition of the specimens and colonoscopy results from patients enrolled into the clinical study is as follows:

- Of the total 24,876 subjects, 1,999 subjects from a prespecified enrollment time window were used toward the device development.
- Of the remaining 22,877 subjects, 10,179 subjects were randomly selected not to be screened with the Shield test. The remaining 12,698 subjects included all CRC subjects and a proportion of non-CRC subjects selected through random down- sampling to match US Census age distribution.
- Of the 12,698 subjects, 10,297 subjects met study inclusion / exclusion criteria and have valid colonoscopy within 183 days and have valid Shield results. This population included 65 subjects with CRC.
- Of the 10,297 subjects, 2,436 were randomly selected for interim specificity analysis and cut-offs selection, therefore, 7,861 subjects were included in pivotal clinical validation dataset.
- The total number of patients in the final clinical validation evaluable dataset consisted of **7,861** subjects with valid colonoscopy and valid Shield test results that were analyzed in the primary analysis dataset.

A sample flowchart for the whole validation dataset is shown in Figure 1 below.

Figure 1. Patient accountability diagram showing breakdown of patient samples and colonoscopy results with sequential application of exclusions between the total available for primary analysis and those not included in the primary analysis.



The percent of Shield Invalid results was 3.1% (345/11,211) with 95%CI: (2.8%, 3.4%). Invalid results were excluded from the data analysis.

Guardant initiated enrollment into the ECLIPSE study on October 8, 2019, with database cutoff on September 30, 2022. The device underwent modifications twice during the course of the study (addition of a protein testing workflow in July 2021 and removal of the same workflow October 2022). The final cfDNA-only device (the Shield test) remained unchanged i.e., the same cfDNA assay workflow, calling algorithms, and classification scores. The final Shield device was locked prior to unblinding of the colonoscopy/biopsy results. The performance reported by Guardant using the final Shield cfDNA cutoff was comparable to a published methodology that enables the use of a pre-specified fixed target for specificity in the primary analysis dataset (Kondratovich et al, 2005). Analyses comparing performance of the primary dataset excluding and including the interim analysis population demonstrated that there were no significant differences in clinical performance observed between subgroups. FDA concluded that the sensitivity and specificity data presented are representative of the device performance.

F. Study Population Demographics and Baseline Parameters

The demographic and baseline characteristics for subjects in the primary analysis dataset considered by Guardant (7,861 subjects constituting final clinical validation evaluable dataset) are presented in Table 12. There was generally a balance of male and female study participants, and the average age was 60 years. 79% of the subjects were White, 12% were Black or African American, and 13% were of Hispanic or Latino. The majority of subjects (70.2%) never smoked.

Table 12. Demographics and Baseline Characteristics of Subjects by Procedural and Lesion Findings

Characteristic	Primary dataset (N=7,861)	CRC (Category 1) (N = 65)	AA (Category 2) (N = 1116)	Non-AN (Category 3-6) (N = 6680)	Non-CRC (Category 2-6) (N = 7796)
Age (years)					
Mean (SD)	60.3 (9.14)	63.2 (8.26)	61.6 (8.67)	60.0 (9.20)	60.3 (9.14)
Median	60	63	62	60	60
Min, Max	45, 84	45, 82	45, 82	45, 84	45, 84
Age Group, n (%)					
45-49	640 (8.1)	4 (6.2)	56 (5.0)	580 (8.7)	636 (8.2)
50-59	3055 (38.9)	13 (20.0)	385 (34.5)	2657 (39.8)	3042 (39.0)
60-69	2440 (31.0)	34 (52.3)	417 (37.4)	1989 (29.8)	2406 (30.9)
70-79	1670 (21.2)	13 (20.0)	252 (22.6)	1405 (21.0)	1657 (21.3)
80+	56 (0.7)	1 (1.5)	6 (0.5)	49 (0.7)	55 (0.7)
Gender, n (%)					
Female	4218 (53.7)	30 (46.2)	511 (45.8)	3677 (55.0)	4188 (53.7)
Male	3643 (46.3)	35 (53.8)	605 (54.2)	3003 (45.0)	3608 (46.3)
Race, n (%)					
American Indian or Alaska Native	14 (0.2)	0	2 (0.2)	12 (0.2)	14 (0.2)
Asian	560 (7.1)	4 (6.2)	56 (5.0)	500 (7.5)	556 (7.1)
Black or African American	931 (11.8)	10 (15.4)	121 (10.8)	800 (12.0)	921 (11.8)
Native Hawaiian or Other Pacific Islander	19 (0.2)	0	2 (0.2)	17 (0.3)	19 (0.2)
White	6167 (78.5)	49 (75.4)	917 (82.2)	5201 (77.9)	6118 (78.5)
Other	137 (1.7)	1 (1.5)	16 (1.4)	120 (1.8)	136 (1.7)
Multiple	23 (0.3)	1 (1.5)	2 (0.2)	20 (0.3)	22 (0.3)
Missing	10 (0.1)	0	0	10 (0.1)	10 (0.1)

Characteristic	Primary dataset (N=7,861)	CRC (Category 1) (N = 65)	AA (Category 2) (N = 1116)	Non-AN (Category 3-6) (N = 6680)	Non-CRC (Category 2-6) (N = 7796)
Ethnicity, n (%)					
Hispanic	1044 (13.3)	11 (16.9)	127 (11.4)	906 (13.6)	1033 (13.3)
Not Hispanic or Latino	6779 (86.2)	54 (83.1)	984 (88.2)	5741 (85.9)	6725 (86.3)
Missing	38 (0.5)	0	5 (0.4)	33 (0.5)	38 (0.5)
BMI category, n (%)					
<30	4610 (58.6)	38 (58.5)	619 (55.5)	3953 (59.2)	4572 (58.6)
>=30 & <35	1873 (23.8)	14 (21.5)	283 (25.4)	1576 (23.6)	1859 (23.8)
35+	1375 (17.5)	13 (20.0)	213 (19.1)	1149 (17.2)	1362 (17.5)
Missing	3 (0.0)	0	1 (0.1)	2 (0.0)	3 (0.0)
Tobacco Use, n (%)					
Never	5522 (70.2)	41 (63.1)	711 (63.7)	4770 (71.4)	5481 (70.3)
Current	737 (9.4)	9 (13.8)	158 (14.2)	570 (8.5)	728 (9.3)
Former	1601 (20.4)	15 (23.1)	247 (22.1)	1339 (20.0)	1586 (20.3)
Missing	1 (0.0)	0	0	1 (0.0)	1 (0.0)
Alcohol Use, n (%)					
Never	3449 (43.9)	30 (46.2)	471 (42.2)	2948 (44.1)	3419 (43.9)
Current	4004 (50.9)	28 (43.1)	583 (52.2)	3393 (50.8)	3976 (51.0)
Former	406 (5.2)	7 (10.8)	62 (5.6)	337 (5.0)	399 (5.1)
Missing	2 (0.0)	0	0	2 (0.0)	2 (0.0)
Illicit Drug Use, n (%)					
Never	7481 (95.2)	63 (96.9)	1052 (94.3)	6366 (95.3)	7418 (95.2)
Current	148 (1.9)	0	26 (2.3)	122 (1.8)	148 (1.9)
Former	229 (2.9)	2 (3.1)	38 (3.4)	189 (2.8)	227 (2.9)
Missing	3 (0.0)	0	0	3 (0.0)	3 (0.0)

G. Safety and Effectiveness Results

1. Safety Results

Adverse effects that occurred in the PMA clinical study:
Of the 43 adverse events reported in subjects who had blood drawn (22,877) from the total enrolled in the ECLIPSE study, 30 (70%) were minor discomfort related to phlebotomy and 13 (30%) were unrelated to the study interventions. No unanticipated adverse device effects (UADEs) were observed across the 22,877 enrolled subjects.

The Shield 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

Shield results could lead to an increased number of colonoscopies and associated adverse events related to the colonoscopy procedure. A false negative Shield result could lead to a colorectal cancer or precancerous lesions remaining undetected.

2. Effectiveness Results

Primary Effectiveness Evaluation

Primary effectiveness evaluation of Shield includes sensitivity for CRC and specificity for AN. Shield clinical performance was evaluated in the primary analysis dataset of 7,861 subjects with valid colonoscopy result and valid Shield test. For the primary objectives, CRC sensitivity was evaluated as the proportion of CRC subjects that had a positive Shield test result; specificity for AN was evaluated as the proportion of subjects without AN that had a negative Shield test result. The results are provided in Table 13.

Table 13. Shield Sensitivity and Specificity

	Colonoscopy/Histopathology			
Shield Result	**CRC (N=65)**	**Non-AN (N=6680)**	**Non-CRC (N=7796)**	**Total (N=7861)**
Positive	54	698	845	899
Negative	11	5982	6951	6962
Total	**65**	**6680**	**7796**	**7861**
CRC Sensitivity = % (n/N) (2-sided 95% Wilson CI)	83.1 (54/65) (72.2, 90.3)			
AN Specificity = % (n/N) (2-sided 95% Wilson CI)	89.6 (5982/6680) (88.8, 90.3)			

Sensitivity for CRC was 83.1%, and specificity for AN was 89.6%.

Secondary Effectiveness Evaluation

The secondary objective of AA sensitivity was evaluated as the proportion of subjects with AA by colonoscopy that had a positive test result in the primary analysis dataset. Sensitivity for AA was 13% (95% CI 11% to 15%).

Table 14. AA sensitivity

Shield Result	**Colonoscopy/Histopathology (Category 2)**
Positive Result	147
Negative Result	969
Total	1116
AA (Category 2) Sensitivity = % (n/N) (2-sided 95% Wilson CI)	13.2 (147/1116)

Age-adjusted Performance measures

The performance of the Shield test is different across age groups (see Table 20 in subgroup analysis section), Because of small sample sizes in the low and high age groups, three age categories were considered: Group 1 (45-59 years), Group 2 (60-69 years) and Group 3 (70+) to evaluate potential differences in the Shield test performance with regard to age.

In the clinical study, an age distribution was different from the age distribution according to 2020 census information. An adjusted analysis for the overall Shield sensitivity for CRC, the overall sensitivity for AA and the overall specificity for non-AN using age grouping performance to assess the distribution of age categories from the US Census population in 2020 is shown in Table 15. The age adjusted performances are not statistically different from the clinical study performance estimates.

Table 15. Age-adjusted performance.

	Age distribution in the Clinical Study data	Age distribution in USA population, 2020
Group 1: 45-59	47.0%	47.8%
Group 2: 60-69	31.0%	29.4%
Group 3: 70+	22.0%	22.8%
	Performance in combined data of clinical study	Age adjusted performance*
Sensitivity for CRC	83.1%	80.8%
Sensitivity for AA	13.2%	12.9%
Specificity for non-AN	89.6%	89.5%

*For example, sensitivities for CRC for group1, 2 and 3 were 76.5%, 88.2% and 78.6% correspondingly. In the US 2020 population, age distribution was 47.8%, 29.4% and 22.8% correspondingly. The prevalence for CRC was 0.30%, 0.43% and 0.64% correspondingly. Then age-adjusted sensitivity for CRC is calculated as (0.478*0.0030*76.5%+0.294*0.0043*88.2%+0.228*0.0064*78.6%)/(0.478*0.0030+0.294*0.0043+0.228*0.0064)=80.8%.

Predictive Values

Analysis was also performed to calculate the positive and negative predictive values (PPV and NPV) for Shield (Table 16).

- The positive predictive value (PPV) for CRC is a fraction of patients with CRC among the patients with positive Shield test results. The PPV for AA is a fraction of patients with AA among the patients with positive Shield test results.
- The negative predictive value (NPV) for CRC is a fraction of patients without CRC among the patients with negative Shield test results. The NPV for AN (CRC or AA) is a fraction of patients without AN among the patients with negative Shield test results.

The PPV for any CRC screening test is impacted by the very low prevalence of CRC in the general population with an average risk (listed in Table 16 below, by age). Unbiased estimates of the CRC prevalence in different age groups were calculated using the data of all subjects enrolled in the ECLIPSE study with colonoscopy results and these unbiased estimates of the CRC prevalence were used in calculation of predictive values presented in

the table below. The age-adjusted CRC prevalence was 0.42%, and the AA prevalence was 10.28%. At this prevalence, the PPV for CRC was 3.10%. The PPV for AA was 12.04%. The NPV for CRC was 99.92% and the NPV for AN was 89.86%.

Note that the prevalence of CRC is increasing with an increase of age from 0.24% in 45-49 age group to 0.96% in 80+ group; the percent of positive Shield results is also increasing with an increase of age from 4.58% in 45-49 age group to 25.81% in 80+ group.

Table 16. PPV and NPV for CRC, AA and AN

	Prevalence of CRC	Prevalence of AA	Percent Positive Shield results	PPV for CRC	PPV for AA	NPV for CRC	NPV for AN
45-49	0.24% (4/1664)	7.39% (123/1664)	4.58%	3.93%	5.76%	99.94%	92.47%
50-59	0.33% (18/5407)	10.49% (567/5407)	7.43%	3.45%	12.09%	99.92%	89.56%
60-69	0.43% (41/9559)	10.78% (1030/9559)	11.11%	3.41%	14.65%	99.94%	89.65%
70-79	0.56% (15/2694)	12.47% (336/2694)	19.41%	2.21%	11.99%	99.84%	87.25%
80+	0.96% (1/104)	6.73% (7/104)	25.81%	3.73%	8.69%	100%	93.95%
Age-adjusted (2020)	**0.42%**	**10.28%**	**11.10%**	**3.10%**	**12.04%**	**99.92%**	**89.86%**

3. Subgroup Analyses

The Shield performance measures in primary analysis dataset were also analyzed according to various demographic characteristics, as well as lesion size and location and key procedural characteristics. There were no pre-specified acceptance criteria for subgroup analyses and the study was not specifically powered for these safety and effectiveness outcomes.

Performance of Shield Test for CRC, AA, and negative findings (non-AN) stratified by lesion covariates in presented in Table 17 below. Results indicate trend toward higher sensitivity with greater lesion size and severity:

- Shield has lower performance of Stage I CRC (54.5%, 12/22; 95%CI 34.7%, 73.1%) than CRC stages 2-4 (100%, 41/41).
- The size of the majority of missed Stage I cancers was less than 10mm.
- Shield did not detect CRC lesions smaller than 10mm (0%, 0/6), 95% CI 0.0% - 39.0%).

Table 17. Clinical performance by lesion covariates (sensitivity and specificity)

Lesion covariates	Category	Sensitivity (n/N)	95% CI
CRC Stage	All	83.1% (54/65)	(72.2%, 90.3%)
	Stage I	54.5% (12/22)	(34.7%, 73.1%)
	Stage II	100.0% (14/14)	(78.5%, 100.0%)
	Stage III	100.0% (18/18)	(82.4%, 100.0%)
	Stage IV	100.0% (9/9)	(70.1%, 100.0%)
	Stage Unknown	50.0% (1/2)	(9.5%, 90.5%)
CRC Lesion Size	All	83.1% (54/65)	(72.2%, 90.3%)
	<5 mm	0.0% (0/1)	(0.0%, 79.3%)
	5-9 mm	0.0% (0/5)	(0.0%, 43.4%)
	10-19 mm	87.5% (7/8)	(52.9%, 97.8%)
	20-29 mm	83.3% (10/12)	(55.2%, 95.3%)
	30+ mm	94.7% (36/38)	(82.7%, 98.5%)
	Unknown	100.0% (1/1)	(20.7%, 100.0%)
AA Lesion Size	All	13.2% (147/1116)	(11.3%, 15.3%)
	<5 mm	0.0% (0/4)	(0.0%, 49.0%)
	5-9 mm	18.8% (9/48)	(10.2%, 31.9%)
	10-19 mm	11.9% (102/859)	(9.9%, 14.2%)
	20-29 mm	13.6% (18/132)	(8.8%, 20.5%)
	30+ mm	23.6% (17/72)	(15.3%, 34.6%)
	Unknown	100.0% (1/1)	(20.7%, 100.0%)
AA Sensitivity Histopathology Sub-categories	All	13.2% (147/1116)	(11.3%, 15.3%)
	Advanced Adenoma, Carcinoma in situ (CIS), any size	0.0% (0/1)	(0.0%, 79.3%)
	Advanced Adenoma, with High-grade dysplasia (HGD), any size	22.6% (7/31)	(11.4%, 39.8%)
	Advanced Adenoma with villous component (>= 25%), any size	17.9% (37/207)	(13.3%, 23.7%)
	Tubular Adenoma >= 10 mm in size	12.0% (82/685)	(9.7%, 14.6%)
	Serrated lesion >= 10 mm in size (includes Sessile serrated adenoma/sessile serrated polyp (SSA/SSP)	11.0% (21/191)	(7.3%, 16.2%)
	Unknown	0.0% (0/1)	(0.0%, 79.3%)
Lesion covariates	**Category**	**Specificity (n/N)**	**95% CI**
Non-AN Specificity Histopathology Sub-categories	All (Categories 3-6)	89.6% (5982/6680)	(88.8%, 90.3%)
	(Category 3) Non-advanced Adenoma, >= 3 adenomas, < 10 mm	87.7% (284/324)	(83.6%, 90.8%)

	(Category 4) Non-advanced Adenoma, 1 or 2 adenomas, > 5 mm, < 10 mm	89.0% (614/690)	(86.4%, 91.1%)
	(Category 5) Non-advanced Adenoma, 1 or 2 adenomas, <= 5 mm	89.1% (1027/1152)	(87.2%, 90.8%)
	(Category 6) Negative, no findings	89.9% (4057/4514)	(89.0%, 90.7%)
CRC Specificity	Categories 2-6 (non-CRC)	89.2% (6951/7796)	(88.5, 89.8)

In addition to AN specificity, specificity for CRC was also evaluated, as the proportion of subjects without CRC that had a negative test result. The estimate of the CRC specificity was 89.2% (6951/7796) with two-sided 95%CI: (88.5%, 89.8%). Please see Table 17 CRC specificity (categories 2-6) above.

The results from subgroup analyses based on demographic and baseline characteristics are shown in Table 18. Shield performance was similar among the subgroups with the exception of two trends. Lower specificity was observed with increased age. Higher AA sensitivity was observed with increased age.

Table 18. Shield Performance by Demographic and Baseline Characteristics

Subgroup	**CRC Sensitivity (N=65) % (n/N)**	**AA Sensitivity (N=1,116) % (n/N)**	**AN Specificity (N=6,680) % (n/N)**
Gender			
Male	80.0 (28/35)	13.1 (79/605)	88.8 (2668/3003)
Female	86.7 (26/30)	13.3 (68/511)	90.1 (3314/3677)
Age Group			
45-49	75.0 (3/4)	3.6 (2/56)	95.5 (554/580)
50-59	76.9 (10/13)	8.6 (33/385)	93.0 (2470/2657)
60-69	88.2 (30/34)	15.1 (63/417)	89.7 (1785/1989)
70-79	76.9 (10/13)	18.7 (47/252)	80.9 (1136/1405)
80+	100.0 (1/1)	33.3 (2/6)	75.5 (37/49)
Race			
American Indian or Alaska Native	(0/0)	0.0 (0/2)	83.3 (10/12)
Asian	75.0 (3/4)	17.9 (10/56)	84.4 (422/500)
Black or African American	90.0 (9/10)	13.2 (16/121)	92.1 (737/800)
Native Hawaiian or Other Pacific Islander	(0/0)	0.0 (0/2)	94.1 (16/17)
White	81.6 (40/49)	13.0 (119/917)	89.8 (4672/5201)
Other	100.0 (1/1)	6.3 (1/16)	84.2 (101/120)
Multiple	100.0 (1/1)	50.0 (1/2)	80.0 (16/20)
Missing	(0/0)	(0/0)	80.0 (8/10)

Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)	AN Specificity (N=6,680) % (n/N)
Ethnicity			
Hispanic or Latino	90.9 (10/11)	18.9 (24/127)	87.3 (791/906)
Not Hispanic or Latino	81.5 (44/54)	12.5 (123/984)	89.9 (5162/5741)
Missing	(0/0)	0.0 (0/5)	87.9 (29/33)
BMI (kg/m^2) at Baseline			
<30	81.6 (31/38)	15.7 (97/619)	88.4 (3494/3953)
≥30 & <35	92.9 (13/14)	8.5 (24/283)	90.2 (1421/1576)
35+	76.9 (10/13)	11.7 (25/213)	92.7 (1065/1149)
Missing	(0/0)	100.0 (1/1)	100.0 (2/2)
Tobacco Use			
Never	82.9 (34/41)	13.4 (95/711)	89.5 (4269/4770)
Current	66.7 (6/9)	11.4 (18/158)	88.4 (504/570)
Former	93.3 (14/15)	13.8 (34/247)	90.2 (1208/1339)
Missing	(0/0)	(0/0)	100.0 (1/1)

Results are additionally presented by key procedural characteristic, lesion location and grade covariates below in Table 19. Shield performance was similar across these covariates.

Table 19. Shield Performance by Procedural Covariates

Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)	AN Specificity (N=6,680) % (n/N)
Bowel Prep			
Excellent	75.0 (12/16)	11.2 (29/259)	89.0 (1646/1849)
Good	85.0 (34/40)	13.0 (93/717)	89.8 (3834/4271)
Fair	100.0 (3/3)	17.9 (25/140)	89.6 (502/560)
Poor	83.3 (5/6)	(0/0)	(0/0)
Most Significant Lesion Location			
Proximal	88.9 (8/9)	14.5 (92/634)	88.7 (1259/1420)
Distal	84.4 (27/32)	10.5 (40/380)	88.8 (556/626)
Rectal	79.2 (19/24)	14.1 (14/99)	91.7 (133/145)
Missing	(0/0)	33.3 (1/3)	89.9 (4034/4489)
Grade			
G1	80.0 (4/5)	N/A	N/A
G2	80.4 (37/46)	N/A	N/A
G3	100.0 (6/6)	N/A	N/A
Missing	87.5 (7/8)	N/A	N/A

4. Pediatric Extrapolation

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

XII. 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 265 principal investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XIII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

N/A

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

A. Panel Meeting Recommendation

A meeting of the Molecular and Clinical Genetics Panel of the Medical Devices Advisory Committee was held on May 23, 2024. The panel voted on the safety, effectiveness, and risk benefit ratio of Guardant Shield.

On Question 1, the panel voted 8 yes, 1 no, 0 abstain that the data shows that there is reasonable assurance that Shield is safe for use in patients who meet the criteria specified in the proposed indication.
On Question 2, the panel voted 6 yes, 3 no, 0 abstain that there is reasonable assurance that Shield is effective for patients who meet the criteria specified in the proposed indication.
On Question 3, the panel voted 7 yes, 2 no, 0 abstain that the benefits of Shield do outweigh the risks for use in patients who meet the criteria specified in the proposed indication.

B. FDA's Post-Panel Action

All panel recommendations are being followed.

The final clinical conditions of approval are cited in the approval order.

1. The following Precaution must be prominently displayed in Shield's labeling and advertising, per 21 CFR 814.82(a)(3):

"Precaution: Based on data from clinical studies, Shield has limited detection (55%-65%) of Stage I colorectal cancer and does not detect 87% of precancerous lesions. One out of 10 patients with a negative Shield result may have a precancer that would have been detected by a screening colonoscopy. Shield demonstrated high detection of Stages II, III, and IV colorectal cancer."

2. Guardant Health Inc. must conduct post-approval study (PAS) listed below and provide the data in PAS reports.

   The Shield Post-Approval Study (PAS) is a prospective, longitudinal study supplemented with Real World Evidence (RWE) to evaluate the longitudinal performance of Shield in an average risk population. If approved, the conditions of approval that will appear in the approval letter include the study requirements meeting the criteria below:

   Study Objective: The study objective is to collect longitudinal data on subjects prescribed Shield over the course of 3 years.

   Study Design: Subjects will be enrolled at T0 and required to complete the Shield test at baseline (T0) and at year 3 (T3). Year 3 is defined as 33 months to 42 months post baseline. Subjects with a positive Shield test result at T0 will undergo diagnostic colonoscopy per the standard of care and then will be discontinued from the study. Subjects with negative Shield test results at T0 will remain in the study. Subjects will be offered repeat Shield testing at T3 and also be expected to undergo a colonoscopy at T3. Subjects will be followed at T1 and T2 to evaluate for changes in medical history.

   Sample size: This study will enroll a sufficient number of subjects at average risk of developing colorectal cancer, age of 45 and 84, to ensure at least 1000 evaluable subjects with valid Shield test results and colonoscopy results at T3 and CRC cases can be observed at T3.

   Study Length: The study length will be 5 years including 3 years of longitudinal subject follow-up.
   - The first subject will be enrolled within 6 months of the study protocol approval date.
   - 20% of subjects enrolled within 12 months of the study protocol approval date.
   - 50% of subjects enrolled within 18 months of the study protocol approval date.
   - 100% of subjects enrolled within 24 months of the study protocol approval date.

   Study Endpoints:
   The post-approval study will include the following co-primary performance measures:
   - Sensitivity for CRC, sensitivity for AA and specificity for non-advanced neoplasia at T3.
   - Positive predictive value (PPV) and negative predictive value (NPV) for CRC, AA and advanced neoplasia at T3.

   The post-approval study will include the following exploratory performance measures:

- The cumulative risk of false positive result (cFPR) and cumulative risk of a true positive result (cTPR).
- The probability that a negative Shield result at baseline remains negative through 3 years.
- The probability that a negative Shield result at baseline (T0) results in no CRC/AA through 3 years.
- The distribution of colorectal epithelial lesions (by Category) among positive Shield subjects at T0 and at T3.
- Adherence to repeat Shield at T3.
- Cumulative compliance to colonoscopy following a positive Shield result.
- Cross-over to alternative screening methodologies (e.g., FOBT, colonoscopy, other) at T1 & T2.
- The rate of no Shield result (e.g., invalid result).
- Supplemental real world evidence study to evaluate PPV at T0 and T3

Interim reporting: FDA tracks and evaluates the conduct of a PAS through review of study reports submitted to the Agency. Guardant health Inc. must fulfill reporting requirements including Enrollment Status Reports, PAS Progress Reports which must be submitted every six months until subject enrollment has been completed, and annually thereafter, as well as a Final Report. Guardant health Inc. must follow the reporting schedule, as required by the PMA approval order, until submitting the Final PAS Report.

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

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

The pivotal clinical study established Shield sensitivity for CRC of 83.1% (95% CI 72.2% to 90.3%), and a specificity for AN of 89.6% (95% CI 88.8% to 90.3%) in average risk population for colorectal cancer. Shield sensitivity for CRC was demonstrated across age groups, racial/ethnic groups, and in both men and women. The observed performance correlations with subject age, CRC stage, and lesion size reflect the known underlying biology of colorectal neoplasia.

In conclusion, the pivotal study demonstrated that Shield met the primary performance point estimate measures for sensitivity and specificity of the study. However, because of the limited detection for Stage I CRC and AA, Guardant will include a precaution in Shield's labeling and advertising and perform post-approval study to gather additional information about benefits and risks of programmatic colorectal cancer screening (i.e., repeated testing over an established period of time).

B. Safety Conclusions

Shield is a non-invasive blood based in vitro diagnostic (IVD) test, and therefore risks associated with the collection of blood sample necessary for the Shield test are minimal. The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above.

There were 43 adverse events reported in the 24,725 subjects who had blood drawn. Of those, 30 (70%) were minor discomfort related to phlebotomy and 13 (30%) were unrelated to the study interventions. No UADEs were observed across the 22,877 enrolled subjects.

With respect to the Shield 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 Shield test is a false positive or false negative result. Since all positive results should lead to a diagnostic colonoscopy, false positive results may lead to patients being referred to colonoscopy unnecessarily. Adverse events commonly associated with colonoscopy include abdominal discomfort and bowel irregularity post-procedure. Rare adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to the sedation resulting in respiratory and/or cardiac events, stroke and death.

In the instance of a false negative Shield result, there is a possibility that a case of colorectal cancer or advanced adenoma could go undetected, which could lead individuals with CRC or AA to forgo other recommended screening procedures such as colonoscopy. Based on the clinical validation data for Shield, there is a chance that as many as 16.9% of patients with CRC, especially stage I CRC and CRC lesions smaller than 10mm, and as many as 86.8% of patients with AA, may be missed by this test, given the current data available. Therefore, it is recommended patients with negative results continue participating in screening programs.

C. Benefit-Risk Determination

Colorectal cancer (CRC) 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. Even with the broadly recognized benefit of CRC screening and the availability of a variety of guideline-recommended screening options, it is estimated that only 68% of eligible individuals are up to date with CRC screening. The Guardant Shield test offers a blood-based alternative to other acceptable screening methodologies, and has probable benefit to the public health, in particular for individuals who are non-compliant with current screening methodologies, and who are more willing to take a blood-based CRC screening test.

The probable benefits of the Guardant Shield test are based on data collected in the ECLIPSE ("Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode") study, which was a prospective, blinded, cross-sectional, study which evaluated the performance of the Shield test as a blood-based screening test for the detection of CRC. The study enrolled a total of 24,876 subjects from 265 sites across the US. Shield clinical performance, for probable benefit, was evaluated in the primary analysis dataset of 7,861 average-risk subjects, age 45 or greater with valid colonoscopy result and valid Shield test. The sensitivity of the Shield test for CRC was 83.1% (54/65) and was only 13.2% (147/1116) for subjects with advanced adenoma (AA). For subjects without a diagnosis of CRC or AA, the specificity of the Shield test was 89.6% (5982/6680). Due to the performance for AA, the indication for this device does not include the detection of AA. In addition, it is clinically valuable to understand the predictive values of this test. Given that the age-adjusted CRC prevalence was 0.42%, and the AA prevalence was 10.28%, the PPV for CRC was 3.10%. The PPV for AA was 12.04%. The NPV for CRC was 99.92% and the NPV for advanced neoplasia (CRC + AA) was 89.86%.

To take a deeper look at the probable benefit, the performance for subgroups was also examined. The sensitivity for Stage I, II, III and IV CRC were 54.5% (12/22), 100.0% (14/14), 100.0% (18/18) and 100.0% (9/9), respectively. Of note, 5 CRC cases in Stage I were not completely staged, and the sensitivity for Stage I may be estimated as 11/17 (64.7%). Also, of note, the size of the majority of missed Stage I cancers was less than 10mm and Shield did not detect CRC lesions smaller than 10mm (0%, 0/6). Also, though the overall sensitivity of Shield for AA was 13.2%, Shield detected 22.6% of AA with high-grade dysplasia and 17.9% of AA with a villous component, both of which represent more aggressive types of AA. Nevertheless, the overall performance of this device for CRC detection indicates probable benefit, in particular for patients non-compliant with CRC screening, or for patients who have barriers to obtaining acceptable CRC screening.

The probable risks associated with the use of this device, are mainly due to: 1) device performance, and 2) diversion from colonoscopy. In terms of device performance, there are key risks related to 1) false positive, 2) false negatives, or 3) failure to provide a result. There is minimal probable risk with the phlebotomy for the use of this device. When used for screening, a positive result should be followed by colonoscopy for diagnosis. A false positive result could result in an additional invasive screening procedure, such as colonoscopy, and thus unnecessarily expose patients to the attendant risks associated with such a procedure. Rare serious adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to sedation. A false negative result with Shield could potentially delay colonoscopy and delay diagnosis of CRC or AA. 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. The sponsor has a post-approval study planned, to demonstrate the probable benefit of follow-up with this test. Another probable risk of this test is diversion to the Shield test, in

lieu of colonoscopy, which more thoroughly detects CRC and AA. To partially mitigate this risk, the provider labeling section on "Patient Education Through Shared Decision-Making" says:

- Colonoscopy should be discussed with all patients, given the test's ability to detect and potentially remove colorectal lesions.

- As part of this decision-making process, it is important to communicate to patients that a negative result does not preclude a CRC or advanced adenoma diagnosis and that they should continue participating in colorectal cancer screening programs. Patients with a positive result should be referred for diagnostic colonoscopy.

To mitigate this risk, patient and provider labeling has been developed, including pictograms of the performance of this test for CRC and AA detection to inform the physician patient encounter; also, a prominent precaution has been placed in the labeling:

- Based on data from clinical studies, Shield has limited detection (55%-65%) of Stage I colorectal cancer and does not detect 87% of precancerous lesions. One out of 10 patients with a negative Shield result may have a precancer that would have been detected by a screening colonoscopy. Shield demonstrated high detection of Stage II, III, and IV colorectal cancer.

To summarize, the key probable risks of this device are the risks associated with device performance (namely sensitivity for Stage I CRC and AA), and risks of diversion from colonoscopy. Also, of note, there are other available modes of screening with better performance than Shield in detecting AA. With adequate physician labeling, a pictogram of device performance for patient-physician shared decision making and prominently placed precautions, the probable risks discussed above are partially mitigated.

Additional factors to be considered in determining probable risks and benefits for the Shield included data from rigorous analytical studies, which demonstrated acceptable analytical performance of this test. Post approval studies are also planned, to validate a testing interval, which may be important in follow-up of negative results. However, the provider labeling clearly says that "A negative result does not preclude a CRC or advanced adenoma diagnosis and that they should continue participating in colorectal cancer screening programs." The risks of a false negative result for CRC or AA are partially mitigated by this labeling, which is intended to inform the shared decision-making process between the physician and patient.

1. Patient Perspective
   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the

PMA for this device.

In conclusion, given the available information above and risk mitigation strategies, the data support that for the detection of CRC, the probable benefits of the Guardant Shield device outweigh the probable risks, in patients who are at average risk of the disease and 45 years or older.

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 ECLIPSE study supports the effectiveness of Shield to screen for the presence of CRC in adults of either sex, 45 years or older, who are average risk for CRC.

XV. CDRH DECISION

CDRH issued an approval order on July 26, 2024.

The final clinical conditions of approval are cited in the approval order and described in Section XIII above.

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

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order and Section XIII above.

XVII. REFERENCES

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