GUARDANT

shield™

Colorectal Cancer Screening Test Instructions for Use

IVD

For in vitro diagnostic use

Rx Only

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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 for 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.

The test is performed at Guardant Health, Inc.

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.

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

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 colorectal cancer.
- Shield has limited ability to prevent the development of colorectal cancer from advanced precancerous lesions and lower detection rates for Stage I colorectal cancer, given the current data available.
    - Shield has lower performance of stage I colorectal cancer [54.5% (12/22); 95% confidence interval (CI) (34.7%, 73.1%)]. The majority (6/10) of missed Stage I cancers were less than 10mm. Shield did not detect colorectal cancer 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 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.
- Colorectal cancer 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 colorectal cancer, 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.
- Patients with an invalid result should be retested or continue participating in colorectal cancer screening programs at the appropriate guideline recommended intervals.
- 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-colorectal 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, for precautions and limitations specific to the collection and shipping of blood samples.

Summary and Explanation of the Test

The Shield test is a blood test developed by Guardant Health for the qualitative detection of colorectal cancer. Shield is a screening test to detect alterations associated with colorectal cancer from whole blood samples collected from individuals at average risk for CRC.

Reagents, Materials, and Equipment

Reagents, materials, and equipment needed to perform the test are used exclusively in the Guardant Health Clinical Laboratory.

The reagents used to perform the Shield assay are listed below:

- 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

The Shield test is intended to be performed with the following instruments 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

The reagents and materials distributed outside of Guardant Health are contained within the blood collection tubes (BCTs) that are part of the Shield Blood Collection Kit (BCK). They consist of the following:

- 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 BCK IFU is included in the Guardant Shield BCK for Cancer Screening.

Principles of the Procedure

The Shield test is a blood test developed by Guardant Health for the qualitative detection of colorectal cancer. Shield is a screening test to detect alterations 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 using next-generation sequencing technology.
The resulting cfDNA data are then analyzed using proprietary bioinformatics algorithms trained to detect the presence of colorectal cancer associated signals. Following final 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.

Test Procedure

Whole Blood Collection and Shipping

The Shield test begins with the collection of whole blood using the Guardant BCK. 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.

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 then 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 were sequenced on the Illumina NovaSeq Sequencing System qualified by Guardant.

Sequencing Data Analysis

Following sequencing, reads are demultiplexed. The methylation partition associated with every molecule is identified by the 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.

Results 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.

The Shield test result is determined based on two scores: the score from a methylation-based regression model (MR model) and the cfDNA integrated score. If either the cfDNA integrated score or the MR score exceeds their respective pre-defined thresholds, the Shield test result is positive. Otherwise, the Shield test result is 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 sequencing where they are analyzed for the right outcome. 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 inprocess and post-sequencing analytical metrics from clinical sample data that are specific and informative to sample performance.

PERFORMANCE CHARACTERISTICS

Non-Clinical Studies

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

A. Analytical Sensitivity

Limit of Detection (LOD)

The LOD was first established as 0.05% estimated tumor fraction (estimated by somatic mutation frequency, max-MAF) using cfDNA from 3 clinical CRC samples diluted with pooled normal samples to create independent pools targeting 6 tumor fraction (MAF) levels from 0.01% to 0.5%. All samples were targeted at a challenging input level that minimally exceeds input QC thresholds.
The 0.05% LOD was verified by testing multiple replicates of 1 AA and 7 CRC sample pools. A 100% positive call rate was observed for 0.05% dilution level and above. These results verify the detection capability at 0.05% dilution level for CRC and AA.

Limit of Blank (LOB)

Samples representing negative Shield test scores were run in 10 different batches, each batch containing a unique combination of two critical reagent lots. Two healthy donor sample pools were assayed across greater than 60 replicates each. A total of 198 replicate samples were assessed. As depicted in Table 1, 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%].

Table 1: Shield LOB

Sample Pool	Number of Replicates	Positive Call Rate [CI]
Pool 1	98	0.00 %
Pool 2	100	3.00%
Total	198	1.52% [0.39%, 4.72%]

B. Precision

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. The samples used to evaluate precision were intended to represent a range of challenging input conditions including high negative, low positive, and borderline (close to the test decision boundary) samples. Precision estimates were obtained in a manner consistent with CLSI document EP05-
A3.[1]

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 4 borderline (close to the test decision boundary) samples. 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 Shield results for the 24 individual samples are shown in Table 2, reflecting the precision primary analysis. The percent of sample replicates called positive for positive and low positive sample categories ranges from 100% to 91.67%. The percent of sample replicates called positive for high negative and negative sample categories ranges from 0% to 17.24%. Discordance in Shield Test Result was observed in samples with Shield Scores near the decision boundary, as expected. Sample mean for the two Shield Scores are also presented per sample.

Table 2: Shield Precision Primary Analysis results per individual sample

Sample Type and ID (A)	Sample Category (B)	Number of Replicates (C)	% Called Positive Shield Test Result (D)	Mean Integrated Score (E)	Mean MR Score (G)
CRC 1	Positive	23	100	3.22	-7.77
CRC 2	Positive	18	100	1.70	-8.19
CRC 3	Positive	23	100	0.39	-8.79
CRC 4	Low Positive	12	91.67	0.91	-10.86
CRC 5	Low Positive	12	100	0.59	-10.03
CRC 6	Low Positive	9	100	0.99	-9.93
CRC 7	Low Positive	28	100	-1.99	-10.25
CRC 8	Low Positive	29	100	-1.65	-9.88
CRC 9	Borderline	4	100	4.01	-7.57

CRC 10	Borderline	12	58.33	-1.36	-11.53
CRC 11	Borderline	17	52.94	-1.61	-11.55
CRC 12	Borderline	12	91.67	-2.05	-10.38
Healthy 1	Borderline	17	11.76	-3.97	-11.54
Healthy 2	High Negative	30	6.67	-3.83	-11.50
Healthy 3	High Negative	30	16.67	-3.18	-11.57
Healthy 4	Negative	30	3.33	-5.02	-11.60
Healthy 5	Negative	30	6.67	-4.58	-11.60
Healthy 6	Negative	36	2.78	-5.61	-11.60
Healthy 7	Negative	29	13.79	-3.82	-11.46
Healthy 8	Negative	29	17.24	-4.03	-11.52
Healthy 9	Negative	34	0	-4.59	-11.70

Sample Type and ID (A)	Sample Category (B)	Number of Replicates (C)	% Called Positive Shield Test Result (D)	Mean Integrated Score (E)	Mean MR Score (G)
Healthy 10	Negative	27	0	-4.13	-11.66
Healthy 11	Negative	29	3.45	-4.37	-11.69
Healthy 12	Negative	16	0	-4.78	-11.79

Sequencer Instrument to Instrument Precision

Following primary sequencing, the same libraries from the six precision batches were sequenced in a secondary sequencer to assess instrument to instrument precision. The results demonstrated high levels of agreement, indicating acceptable sequencer-to-sequencer precision performance.

Batch Pooling Tolerance

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 results demonstrated high levels of agreement, indicating acceptable tolerance to batch pooling strategy for the Shield Test.

C. Interfering Substances

Whole blood contains endogenous substances that could potentially interfere with an assay when present at elevated levels. To evaluate these potential interferences, pooled clinical positive samples and screened negative samples were spiked with elevated concentrations of interfering substances at levels recommended in the CLSI guidelines EP07-ED3[2] and EP37-EDI[3] (Table 3). The endogenous interfering substances evaluated were unconjugated bilirubin, conjugated bilirubin, triglycerides, genomic DNA, albumin, and hemoglobin. No interference with the Shield test was observed for any of the substances at the concentrations tested.

Table 3: 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

D. Cross-reactivity with non-CRC Cancers and Other Diseases

Non-CRC Cancers

The potential for cross-reactivity with non-colorectal cancers was evaluated in 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 nonCRC cancer. The number of samples evaluated for each cancer type is shown in Table 9. 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 4 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 4: Cross-reactivity in the context of Cancer Incident Rates

Cancer Type	Incidence in US in 2022[4]	Incident Rate per 10,000 individuals	Positive Rate of Shield test	Number of positive calls in 10,000 subjects
Bladder	81,180	2.44	85.2%	2.07
Breast	290,560	8.74	80.0%	6.99
Gastric*	47,020	1.41	92.3%	1.30
Kidney	79,000	2.34	50.0%	1.17
Liver	41,260	1.24	87.5%	1.09
Lung	236,740	7.12	76.5%	5.44

Melanoma	99,780	3.00	57.1%	1.71
Ovarian	19,880	0.60	61.5%	0.37
Pancreatic	62,210	1.87	92.7%	1.73
Prostate	268,490	8.07	63.2%	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 rates were evaluated in combination with the disease prevalence observed in the interim analysis dataset in ECLIPSE, to assess the impact of disease on Shield specificity. The results are shown in Table 5 below.

Table 5: Non-Cancer Cross-Reactivity Results

Disease	Number of valid samples	Prevalence Observed in Interim Analysis Set	Positivity Rate	Projected number of positive Shield calls in 10,000 subjects
Hypertension	569	23.3%	10.5%	245.58
Disease	Number of valid samples	Prevalence Observed in Interim Analysis Set	Positivity Rate	Projected number of positive Shield calls in 10,000 subjects
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

E. Cross-Contamination / Carry-Over

Cross-contamination and carry-over were evaluated with a checkerboard design alternating precharacterized high positive donor samples and low negative donor samples in two plates processed consecutively using one single line of instruments. A total of 47 sample replicates of each type were tested in 2 batches for a total of 188 replicates.
The PPA and NPA for first batch is 100% and the PPA and NPA for the second batch is 100% and 97.83%, respectively. One false positive Shield test result was observed 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 Guardbanding

Guardbanding studies were performed to establish the tolerance of the Shield assay to variations in critical assay workflow parameters in the categories listed below:

1. Reagent Volumes relative to instrument tolerances for key reagents
2. Incubation Times including library enrichment and cfDNA hybridization times
3. Hold Point durations for extracted cfDNA and samples during processing
4. Temperature variations during the enrichment hybridization process

Samples 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. A minimum of 6 positive and 6 negative samples were tested per guardbanded condition and compared to reference condition. A total of 845 sample replicates were processed for these studies. PPA for the overall Shield call observed across all conditions tested was 100%, and NPA was 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 variations were acceptable with respect to assay performance.

Input Guardbanding

An input guardbanding study was conducted to evaluate the robustness of the Shield assay at different input levels. The study aimed to assess the assay's performance at the low cfDNA input corresponding to performance above and below the quality control (QC) cutoff levels, ensuring reliable results even with low cfDNA input.

Each run involved testing multiple samples, including positive and negative samples, at 4 challenging input levels including samples with scores near the clinical decision cutoff. A total of 186 sample replicates were processed through the Shield assay workflow.

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 Studies On-Board

Stability

The purpose of the on-board stability study 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 sample were tested in 1624 replicates per condition to evaluate the tolerance of the Shield assay to the final onboard hold conditions tested. Test condition samples were compared with reference condition samples and showed no significant difference in assay performance. Samples were processed with the Shield workflow with the following hold points and were shown to be stable for the stated hold times:

1. Shield reagents are stable for at least 30 minutes at room temperature on the Hamilton Microlab STAR deck.
2. Samples are stable at 2-8℃ 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:

1. Samples stored after Library Prep Clean-up at -15 to -25℃ for 20 days with one freeze-thaw cycle
2. Samples stored after Enrichment Transfer at -15 to -25℃ for 14 days with one freeze-thaw cycle
3. Samples stored after Sequencing Normalization at -15 to -25℃ 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.

Reagent Shelf-Life Stability

The stability of reagents was evaluated following guidance from CLSI standard: EP25-A (Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline).

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℃ 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. At each time point, twenty replicates of Shield positive and negative samples were tested.

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 at least 12 months. 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.

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 total of 520 sample replicates were processed for this study, and classification calls were compared between reference and storage conditions. No significant difference in Shield integrated score and MR score was observed at different time-points for each sample transport and storage condition tested, demonstrating that whole blood samples stored and shipped in Guardant BCTs are stable for up to 9 days at room temperature.

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 (12-15 CRC samples per time-point) and 2-3 freeze-thaw cycles. A total of 344 sample replicates were processed for this study.

This study established the short-term stability of plasma isolated from BCTs for 30 days with 2 freezethaws and 15 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 236 sample replicates have been processed 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 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. At each time point, twenty replicates of Shield positive and negative samples were tested. 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.

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 bloodbased 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 summary of the clinical study design and results is provided below.

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 of average risk for CRC. Patients eligible for colon cancer screening and intending to undergo colonoscopy were enrolled in the study. To evaluate the performance characteristics of Shield, the test results were compared with the findings of the

colonoscopy. 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 Shield blood collection kits from all enrolled subjects prior to the patient undergoing standard of care colonoscopy and any associated bowel preparation. The performance of the Shield test was compared against the 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 listed in Table 6. A total of 24,876 patients were enrolled from October 2019 to September 30, 2022. There were 265 participating clinical sites, with the Shield test being performed internally at Guardant Health.

Table 6: Colonoscopy/Histopathology Diagnosis Category Descriptions

Category	Findings
1	Colorectal cancer, any stage
2	Advanced adenoma
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
4	Non-advanced adenoma, 1 or 2 adenomas, >5 mm, <10 mm
5	Non-advanced adenoma, 1 or 2 adenomas, ≤5 mm
Category	Findings
6	Negative colonoscopy, or other findings
7	Not evaluable

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the ECLIPSE study was limited to patients 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.

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

- 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.

2. Follow-up Schedule

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

3. Clinical Endpoints

The primary objective of this study was to establish the performance characteristics of the Shield test sensitivity for CRC (category 1, Table 6) and specificity of advanced neoplasia (categories 3, 4, 5, and 6, Table 6) in average-risk patients against the clinical results defined by colonoscopy/histopathology diagnosis.

The secondary objective included establishing the sensitivity of the Shield test in the detection of advanced adenomas in average-risk patients.

B. Accountability of PMA Clinical Validation Dataset

Samples were collected from a total of 24,876 subjects at 265 sites for the Shield test. Of those, 1,999 were included in the development dataset, leaving 22,877 enrolled in the clinical validation dataset. A further 12,619 were not selected due to the protocol specified sampling plan or inclusion in other analysis datasets. Of the remaining 10,258 subjects selected for clinical validation, 2,397 were not eligible for inclusion in the coprimary and secondary analyses. The final clinical validation (CV) dataset included 7,861 enrolled subjects.

C. Study Population Demographics and Baseline Parameters

The demographic and baseline characteristics for subjects in the clinical validation dataset are presented in Table 7. 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 Hispanic or Latino. The majority of subjects (70.2%) never smoked.

Table 7: Demographics and Baseline Characteristics of Subjects by Procedural and Lesion Findings

Characteristic	CV Dataset (N=7,861) n (%)	CRC (Category 1) (N = 65) n (%)	AA (Category 2) (N = 1116) n (%)	Non-CRC (Category 2-6) (N = 7796) n (%)	Non-AN (Category 3-6) (N = 6680) n (%)
Age (years)					
n	7861	65	1116	7796	6680
Mean (SD)	60.3 (9.14)	63.2 (8.26)	61.6 (8.67)	60.3 (9.14)	60.0 (9.20)
Median	60	63	62	60	60
Min, Max	45, 84	45, 82	45, 82	45, 84	45, 84
Age Group					

Characteristic	CV Dataset (N=7,861) n (%)	CRC (Category 1) (N = 65) n (%)	AA (Category 2) (N = 1116) n (%)	Non-CRC (Category 2-6) (N = 7796) n (%)	Non-AN (Category 3-6) (N = 6680) n (%)
45-49	640 (8.1)	4 (6.2)	56 (5.0)	636 (8.2)	580 (8.7)
50-59	3055 (38.9)	13 (20.0)	385 (34.5)	3042 (39.0)	2657 (39.8)
60-69	2440 (31.0)	34 (52.3)	417 (37.4)	2406 (30.9)	1989 (29.8)
70-79	1670 (21.2)	13 (20.0)	252 (22.6)	1657 (21.3)	1405 (21.0)
80+	56 (0.7)	1 (1.5)	6 (0.5)	55 (0.7)	49 (0.7)
Gender, n (%)					
Female	4218 (53.7)	30 (46.2)	511 (45.8)	4188 (53.7)	3677 (55.0)
Male	3643 (46.3)	35 (53.8)	605 (54.2)	3608 (46.3)	3003 (45.0)
Race, n (%)					
American Indian or Alaska Native	14 (0.2)	0	2 (0.2)	14 (0.2)	12 (0.2)
Asian	560 (7.1)	4 (6.2)	56 (5.0)	556 (7.1)	500 (7.5)
Black or African American	931 (11.8)	10 (15.4)	121 (10.8)	921 (11.8)	800 (12.0)

Native Hawaiian or Other Pacific Islander	19 (0.2)	0	2 (0.2)	19 (0.2)	17 (0.3)
White	6167 (78.5)	49 (75.4)	917 (82.2)	6118 (78.5)	5201 (77.9)
Other	137 (1.7)	1 (1.5)	16 (1.4)	136 (1.7)	120 (1.8)
Multiple	23 (0.3)	1 (1.5)	2 (0.2)	22 (0.3)	20 (0.3)

Characteristic	CV Dataset (N=7,861) n (%)	CRC (Category 1) (N = 65) n (%)	AA (Category 2) (N = 1116) n (%)	Non-CRC (Category 2-6) (N = 7796) n (%)	Non-AN (Category 3-6) (N = 6680) n (%)
Missing	10 (0.1)	0	0	10 (0.1)	10 (0.1)
Ethnicity, n (%)					
Hispanic	1044 (13.3)	11 (16.9)	127 (11.4)	1033 (13.3)	906 (13.6)
Not Hispanic or Latino	6779 (86.2)	54 (83.1)	984 (88.2)	6725 (86.3)	5741 (85.9)
Missing	38 (0.5)	0	5 (0.4)	38 (0.5)	33 (0.5)
BMI category, n (%)					
<30	4610 (58.6)	38 (58.5)	619 (55.5)	4572 (58.6)	3953 (59.2)
>=30 & <35	1873 (23.8)	14 (21.5)	283 (25.4)	1859 (23.8)	1576 (23.6)

35+	1375 (17.5)	13 (20.0)	213 (19.1)	1362 (17.5)	1149 (17.2)
Missing	3 (0.0)	0	1 (0.1)	3 (0.0)	2 (0.0)
Tobacco Use, n (%)					
Never	5522 (70.2)	41 (63.1)	711 (63.7)	5481 (70.3)	4770 (71.4)
Current	737 (9.4)	9 (13.8)	158 (14.2)	728 (9.3)	570 (8.5)
Former	1601 (20.4)	15 (23.1)	247 (22.1)	1586 (20.3)	1339 (20.0)
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)	3419 (43.9)	2948 (44.1)
Current	4004 (50.9)	28 (43.1)	583 (52.2)	3976 (51.0)	3393 (50.8)
Former	406 (5.2)	7 (10.8)	62 (5.6)	399 (5.1)	337 (5.0)
Characteristic	CV Dataset (N=7,861) n (%)	CRC (Category 1) (N = 65) n (%)	AA (Category 2) (N = 1116) n (%)	Non-CRC (Category 2-6) (N = 7796) n (%)	Non-AN (Category 3-6) (N = 6680) n (%)
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)	7418 (95.2)	6366 (95.3)
Current	148 (1.9)	0	26 (2.3)	148 (1.9)	122 (1.8)

Former	229 (2.9)	2 (3.1)	38 (3.4)	227 (2.9)	189 (2.8)
Missing	3 (0.0)	0	0	3 (0.0)	3 (0.0)

D. 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, which strongly supports the safety of the Shield device.

2. Effectiveness Results

Primary Objectives:

Shield clinical performance is based on the evaluable set of 7,861 subjects with valid colonoscopy diagnosis and valid test results. For the primary objectives, CRC sensitivity (Category 1) was evaluated as the proportion of CRC subjects that had a positive test result; and specificity for AN (Category 3-6) was evaluated as the proportion of subjects without AN that had a negative test result. These results are provided in Table 8.

Table 8: Distribution of Shield Result by Histological Category - Primary Objective

Shield Result	CRC (Category 1)	AN Specificity (Categories 3-6)
Positive	54 (83.1%)	698 (10.4%)
Negative	11 (16.9%)	5982 (89.6%)
Total	65	6680
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 (5,982/6,680) (88.8%–90.3%)

The primary objectives of the ECLIPSE study were demonstration of greater than 65% lower bound of the 2-sided 95% confidence interval for CRC (Category 1) and greater than 85% lower bound of the 2sided confidence interval for AN (Categories 3-6). Shield sensitivity for CRC was 83.1% (54/65) with a two-sided 95% confidence interval of 72.2% to 90.3% (lower bound of 72.2%) (Table 8). Shield specificity for AN was 86.6%, with a two-sided 95% confidence interval of 88.8% to 90.3% (lower bound of 88.8%) (Tables 8, 9).

Table 9: Specificity Analysis

Case Category Specificity	n/N (%) (95% CI)
3: Non-advanced adenoma, >3 adenomas, <10 mm	284/324 (87.7%) (83.6, 90.8)
4: Non-advanced adenoma, 1 or 2 adenomas, >5 mm, <10 mm	614/690 (89.0%) (86.4, 91.1)
5: Non-advanced adenoma, 1 or 2 adenomas, ≤5 mm	1027/1152 (89.1%) (87.2, 90.8)
6: Negative colonoscopy, or other findings	4057/4514 (89.9%) (89.0, 90.7%)
Categories 2-6	6951/7796 (89.2%) (88.5, 89.8)
Categories 3-6	5982/6680 (89.6%) (88.8, 90.3)

Secondary Objectives:

The secondary objective of AA sensitivity was evaluated as the proportion of the clinical validation dataset subjects with AA (category 2) by colonoscopy that had a positive test result. Sensitivity for Category 2 (AA) was 13.2% (95% CI 11.3% to 15.3%; Table 10).

Table 10: Category 2 (Advanced Adenoma) Sensitivity - Secondary Objective

Shield Result	Category 2 (N=1116)
Positive Result	147
Negative Result	969
Total	1116
AA (Category 2) Sensitivity = % (n/N) (2-sided 95% Wilson CI)	13.2 (147/1116) (11.3, 15.3)

Exploratory Objectives:

Among all participants who enrolled in the clinical Validation dataset of ECLIPSE, met inclusion / exclusion criteria, and had valid histopathological results, the CRC prevalence was 0.41%, and the AN prevalence was 11.2%. At this prevalence, the positive predictive value for CRC was 3.03% (95% CI: 2.7%–3.4%). The CRC positive likelihood ratio is 7.5. The positive predictive value for AN was 17.0% (95% CI: 15.0%–19.1%). The negative predictive value for CRC was 99.9% (95% CI: 99.9% - 100%).

Specificity for the absence of any colorectal neoplasia (negative colonoscopy or other findings, Category 6) was calculated as 89.9% (95% CI 89.0 - 90.7) (Table 9).

Subgroup Analyses:

The results from subgroup analyses based on demographic and baseline characteristics are shown in Table 11. Results by key procedural and lesion characteristics are in Table 12.

Table 11: Device 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)

Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)	AN Specificity (N=6,680) % (n/N)
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)
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)
Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)	AN Specificity (N=6,680) % (n/N)
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)

Table 12: Device Performance by Procedural and Lesion Covariates

Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)
CRC Stage		
I*	54.5 (12/22)	N/A

II	100.0 (14/14)	N/A
III	100.0 (18/18)	N/A
IV	100.0 (9/9)	N/A
Stage Unknown	50.0 (1/2))	N/A
I-III	81.5 (44/54)	N/A
Lesion Size (mm)		
<5 mm	0.0 (0/1)	0.0 (0/4)
5-9 mm	0.0 (0/5)	18.8 (9/48)
10-19 mm	87.5 (7/8)	11.9 (102/859)
20-29 mm	83.3(10/12)	13.6 (18/132)
Subgroup	CRC Sensitivity (N=65) % (n/N)	AA Sensitivity (N=1,116) % (n/N)
30+ mm	94.7 (36/38)	23.6 (17/72)
Missing	100.0 (1/1)	100.0 (1/1)
AA Sensitivity Histopathology Diagnosis Sub-categories		
Advanced Adenoma, carcinoma in situ, any size (Category 2a)	N/A	0.0 (0/1)
Advanced Adenoma, high-grade dysplasia, any size (Category 2b)	N/A	22.6 (7/31)
Advanced Adenoma, villous component (≥25%), any size (Category 2c)	N/A	17.9 (37/207)

Tubular Adenoma ≥10 mm in size (Category 2d)	N/A	12.0 (82/685)
Serrated lesion ≥10 mm in size (Category 2e)	N/A	11.0 (21/191)
Most Significant Lesion Location		
Proximal	88.9 (8/9)	14.5 (92/634)
Distal	84.4 (27/32)	10.5 (40/380)
Rectal	79.2 (19/24)	14.1 (14/99)
Missing	(0/0)	33.3 (1/3)
Grade		
G1	80.0 (4/5)	N/A
G2	80.4 (37/46)	N/A
G3	100.0 (6/6)	N/A
Missing	87.5 (7/8)	N/A

*Assumes 5 incompletely staged by AJCC malignant polyps are Stage I disease.

PEDIATRIC EXTRAPOLATION

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

MEANING OF SYMBOLS

	Sterilized Using Irradiation
	Catalog Number
	Use By
	Serial Number
	Batch Code
	In Vitro Diagnostic Medical Device
	Content Sufficient for Number of Tests Specified
	Do Not Re-use
	Biological Risks
	Consult Instructions for Use
	Temperature Limit

	Manufacturer
Rx Only	By Prescription Only

TRADEMARKS

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