Freenome

SimpleScreen™ CRC

Colorectal Cancer Screening Blood Test

Instructions for Use

IVD

For in vitro diagnostic use

Rx Only

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CONTENTS

INTENDED USE

Intended Use:
The SimpleScreen™ CRC test is a qualitative, in vitro diagnostic test intended to detect molecular signals associated with colorectal cancer. SimpleScreen CRC utilizes circulating cell-free DNA from plasma of peripheral whole blood collected in the SimpleScreen CRC blood collection kit.

SimpleScreen CRC is indicated for colorectal cancer screening in adults aged 45 years and older who are at average risk for the disease. Patients with a positive result should be followed by colonoscopy. SimpleScreen CRC is not a replacement for diagnostic colonoscopy or for surveillance colonoscopy in high-risk individuals.

PRECAUTION

Based on data from clinical studies, SimpleScreen CRC has limited detection (57%) of Stage I colorectal cancer (64% when U.S. 2020 Census age-sex adjusted) and does not detect 87% of advanced precancerous lesions (86% when U.S. 2020 Census age-sex adjusted). One out of 10 patients with a negative SimpleScreen CRC result may have a precancer that would have been detected by a screening colonoscopy.

CONTRAINDICATIONS

SimpleScreen CRC is NOT indicated for use for patients that have the following:

- Personal history of colorectal cancer (CRC), advanced precancerous lesions (APLs), or other related cancers
- Family history of CRC, defined as having one or more first-degree relatives (parent, sibling, or child) diagnosed with CRC at any age
- Positive result on another CRC screening method within the last six months, or:
    - A fecal occult blood test (FOBT) or a fecal immunochemical test (FIT) within the last 12 months
    - A FIT-DNA test within the last 36 months
- Personal history of any of the following high-risk conditions for CRC:
    - Inflammatory bowel disease (IBD), including chronic ulcerative colitis (CUC) and Crohn's disease
    - Relevant hereditary cancer syndromes including but not limited to: hereditary non-polyposis colorectal cancer syndrome (HNPCC) or Lynch syndrome, familial

adenomatous polyposis (FAP or Gardner's), Familial Hyperplastic Polyposis, Peutz-Jeghers syndrome, MUTYH-associated polyposis (MAP), Cowden's syndrome, Juvenile polyposis syndrome, serrated polyposis syndrome, Turcot's/Crail's syndrome, Cronkhite-Canada syndrome, or Neurofibromatosis

LIMITATIONS

- Providers should discuss the most appropriate screening test to use with patients depending on their medical history and individual circumstances. SimpleScreen CRC is not intended as a screening test for individuals who are at high risk for colorectal cancer (CRC).
- SimpleScreen CRC has limited ability to prevent the development of colorectal cancer from advanced precancerous lesions (APLs) and has lower detection rates for Stage I CRC, given the current data available.
    - SimpleScreen CRC has lower performance of Stage I CRC [57.1% (16/28); 95% CI (39.1%-73.5%)].
    - SimpleScreen CRC may fail to detect as many as 87.5% of patients with advanced precancerous lesions which can later become neoplastic because of its limited detection of advanced precancerous lesions. [12.5% (321/2567); 95% CI (11.3%-13.8%)].
- SimpleScreen CRC may produce false positive or false negative results.
    - A false positive result occurs when the test produces a positive result even though a colonoscopy will not find colorectal cancer or advanced precancerous lesions. SimpleScreen CRC has a false positive rate of 10%, meaning 1 of 10 people who do not have advanced colorectal neoplasia (colorectal cancer or advanced precancerous lesions) will have a false positive test result.
    - A false negative result occurs when the test does not detect colorectal cancer or advanced precancerous lesions although a colonoscopy would identify either of these findings. SimpleScreen CRC has a false negative rate of 21% for colorectal cancer, meaning 21 of 100 people who have colorectal cancer will incorrectly have a negative test result.
- A positive SimpleScreen CRC test result suggests patients may have colorectal cancer or advanced precancerous lesions. Patients with a positive result should be followed by colonoscopy.
- A negative SimpleScreen CRC test result does not guarantee absence of colorectal cancer or advanced precancerous lesions. Patients with a negative result should continue participating in colorectal cancer screening programs, at the appropriate

guideline-recommended intervals.

  - One out of 1600 patients testing negative will be falsely reassured that they are negative for colorectal cancer, given the negative predictive value for colorectal cancer of 99.9%.
  - One out of 10 patients testing negative will be falsely reassured that they are negative for advanced precancerous lesions, given the negative predictive value for advanced precancerous lesions of 91%.

- 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.
- The performance of SimpleScreen CRC has been established in a prospective, cross-sectional study with consecutively enrolled participants. The benefits and risks of programmatic colorectal screening (i.e., repeated testing over an established period of time) with SimpleScreen CRC have not been studied.
- The prospective, cross-sectional study cohort may have included participants with one first-degree relative diagnosed with colorectal cancer after age 60.
- Non-inferiority or superiority of SimpleScreen CRC sensitivity as compared to other recommended screening methods for colorectal cancer or advanced precancerous lesions 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, and prostate cancers.
- Consult the Freenome Blood Collection Kit (BCK) Instructions for Use for precautions and limitations specific to the collection and shipping of blood samples.

SUMMARY AND EXPLANATION OF TEST

SimpleScreen CRC is a qualitative in vitro diagnostic (IVD) test used to detect molecular signals (methylation) associated with colorectal cancer (CRC) from whole blood samples collected from individuals at average risk for the disease. A whole blood sample is collected by licensed healthcare providers using the Freenome CRC Blood Collection Kit (BCK) and is then shipped to Freenome's CLIA-certified clinical laboratory for testing.

SimpleScreen CRC requires a standard blood draw that can be completed at any patient visit, on its own or alongside other blood tests. SimpleScreen CRC does not require any dietary or

medication restrictions before blood is drawn. For details about collecting and shipping blood samples to the Freenome Clinical Laboratory, refer to the Freenome BCK Instructions for Use.

Once laboratory processing of the blood samples is completed, molecular data are analyzed by a proprietary software platform. Test results are delivered back to the ordering physician in about two weeks.

SimpleScreen CRC yields a result that is “Positive” or “Negative.” A positive result indicates a signal was detected that may suggest the presence of colorectal cancer or advanced precancerous lesions. Patients with a positive result should be followed by colonoscopy. A negative result indicates the test did not detect a signal associated with CRC or APLs. A negative test result does not guarantee absence of CRC or APLs. Patients with a negative result should continue participating in colorectal cancer screening programs, at the appropriate guideline-recommended intervals. In some cases, SimpleScreen CRC may generate a “No Result.” If this happens, the ordering physician will be contacted, and the patient may be asked to provide another blood sample.

Reagents, Materials, and Equipment

Reagents, materials, and equipment needed to perform the SimpleScreen CRC test are used exclusively in the Freenome clinical laboratory. The only components distributed outside of Freenome are contained within the Freenome BCK. The following components are required to perform the SimpleScreen CRC test:

Blood Collection Kit:
The BCK (catalogue number K00019) comprises all components used for the collection, stabilization, packaging, and transportation of whole blood samples. The BCK contains two BCTs and packaging materials with instructions for storage, sample collection, and shipping contents to the Freenome clinical laboratory.

- Streck cfDNA BCTs (2)
- BCT labels
- Patient Information Sheet
- BCK Instructions for Use (catalogue number K00014)
- Biohazard bag
- Foil pouch
- Absorbent sleeve
- Gel pack
- Shipping label and envelope

Assay:
The following reagents are required to perform the SimpleScreen CRC test and are qualified by Freenome under the Freenome Quality System:

- cfDNA extraction kit
- Library preparation kit
- DNA quantification kit
- Next generation sequencing kit and manufacturer-provided sequencer control

Instrumentation:
The following instruments are required to perform the SimpleScreen CRC test and are qualified by Freenome under the Freenome Quality System:

- Hamilton Microlab easyBlood STARlet instrument
- Thermo Fisher Qubit® Flourometer
- Hamilton Microlab STAR instrument
- Molecular Devices SpectraMax® Plate Reader
- Agilent Bravo instrument
- Illumina NovaSeq™ 6000

Software:
SimpleScreen CRC includes software used by Freenome to process data from the patient's sample and return a test report to the physician. The Freenome Software Platform uses the sequencing data from the samples processed in the laboratory. These sequencing data are transformed and processed by an AI/ML-enabled classification model which produces a score and compares it to a pre-defined threshold in order to generate a binary result that is included in a test report.

TEST PROCEDURE

The overall SimpleScreen CRC test is comprised of the following elements:

- Blood Collection Kit
- Pre-Analytical Process
- Genomics Workflow
- Freenome Software Platform

Sample Collection and Shipping using the Blood Collection Kit
The initial step of the SimpleScreen CRC test is the collection of whole blood in two Streck cfDNA BCTs that are provided as part of the BCK. The BCK enables whole blood collection and preservation of cfDNA for downstream analytical processing. The whole blood specimen is collected from the individual by licensed healthcare providers and shipped at room temperature

to Freenome where samples are received and accessioned.

Prior to blood draw, the Freenome 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.

Pre-Analytical Process
After accessioning, plasma collected from the BCTs is separated from the whole blood using automated instrumentation. The plasma is then aliquoted and frozen for future analytical processing. Whole blood specimens must be processed within 7 days of blood collection.

Genomics Workflow
The genomics workflow begins with 2 mL of plasma and assay controls which are plated together for cfDNA extraction. After the extraction process, the extraction control concentration is quantified as an in-process quality control (QC) checkpoint. Samples then proceed to library preparation.

Reagents are prepared prior to the associated library preparation steps. Reagent preparation includes the preparation of reagent master mixes used in library preparation, as well as preparation of the quantification reagent used for in-process QC steps. Library preparation includes end repair, adapter ligation, unmethylated cytosine conversion, and polymerase chain reaction (PCR) steps.

After PCR, prepared libraries are quantified for multiplexing and as an in-process QC checkpoint. Next, the multiplexing process pools a portion of each of the libraries and controls from a single plate together into an individual plate library pool, concentrates the pool, and adds the prerequisite reagents for target enrichment.

Target enrichment is then performed, followed by PCR amplification in order to ensure libraries are of sufficient concentration for sequencing. This is confirmed by a quantification step as another in-process QC checkpoint and for normalization and pooling.

After pooling, each individual library pool is then quantified as an in-process QC checkpoint. A combined library pool can then be created from up to four individual plate pools using the quantification of each individual plate library pool. If a combined library pool was created, it is then quantified as an additional in-process QC checkpoint to ensure sufficient pooled library mass is present for sequencing.

Sequencing is then performed and, when complete, the sequencing data are transferred to a

data storage location for downstream processing by the software platform.

Analysis and Results Reporting
The SimpleScreen CRC test includes proprietary cloud-based software used for analytical pre-processing and computational transformation of sequencing data, application of the trained AI/ML classification algorithm, and test report generation.

Analytical pre-processing of the sequencing data from laboratory-processed samples includes demultiplexing; sequence alignment; QC assessment of the positive control, negative control, and no template control; and sample QC. After analytical pre-processing, the pre-processed sequencing data are computationally transformed to produce the structured quantitative values used as inputs to the AI/ML-enabled classification model. The AI/ML-enabled classification model uses this information to identify hypermethylated fragments (HMFs) across multiple regions of the genome, then aggregates information from all loci with a non-linear integrative function to generate a score.

The score is compared to a threshold, yielding a qualitative result. The learned classification threshold was selected to yield a desired specificity, based on behavior in negative samples during training. If the score is greater than or equal to the threshold, the classifier returns a positive call; if the score is less than the threshold, it returns a negative call.

Based on the call from the AI/ML classification model, a test report is generated with a diagnostic result that can be either “Positive” or “Negative” for the sample. In some cases, the SimpleScreen CRC may generate a “No Result” for a sample.

Quality Control Measures

SimpleScreen CRC utilizes a number of controls as part of the genomics workflow.

- The Positive Control (PC) contains sheared genomic DNA from a cell line with a known HMF rate similar to early-stage CRC samples.
- The Negative Control (NC) contains PCR-amplified sheared genomic DNA from a cell line that should yield a negative result (PCR amplification produces effectively unmethylated DNA).
- The Extraction Control (EC) is used to assess cfDNA extraction success and is then removed from further processing.
- The No-Template Control (NTC) is used to detect nucleic acid contamination in assay reagents, consumables, or instruments.

The controls are treated as individual samples in the workflow. As QC measures, the PC must meet a range criteria while the NC and NTC must fall below respective thresholds for reporting

valid patient test results. If there is a QC failure during any of these steps, a “No Result” test report is generated for the sample.

PERFORMANCE CHARACTERISTICS

Non-Clinical Studies

Non-clinical studies were conducted at Freenome, LLC to evaluate the analytical performance of SimpleScreen CRC. The studies are described below.

A. Analytical Sensitivity

Limit of Detection (C95 Establishment)

The Limit of Detection (LoD) for SimpleScreen CRC was defined as the system score (S) where the test is expected to declare a sample to be positive 95% of the time (also referred to as the C95). To establish the C95 (LoD), 18 clinical blend samples (3 clinical donors × 6 target hypermethylated fragments levels ($4.00x10^{-4}$, $2.00x10^{-4}$, $1.00x10^{-4}$, $5.00x10^{-5}$, $2.50x10^{-5}$, and $1.25x10^{-5}$) per donor) were tested across 2 reagent lots with 48 replicates per sample for each lot. All batches began with 4 ng cfDNA input into library preparation, an input mass below the fifth percentile of the 95% intended use population. Testing for each reagent lot included 3 processing runs, 4 batches per processing run, and 4 replicates per sample for each batch. In total, 1728 replicates were processed, of which 1698 passed QC and were used to establish the C95 (LoD).

The C95 (LoD) was determined for each reagent lot by fitting a probit model to the % of replicates positive for each sample (hit rate) as the outcome and the corresponding mean system score (S) as the predictor variable. The C95 (LoD) for each reagent lot was determined as the S value corresponding to 95% positive call rate (**Table 1).**

Table 1: C95 Values per Reagent Lot

Lot	Calculated C95 (LoD)
1	0.712085
2	0.736821

C95 = limit of detection (LoD)

The C95 (LoD) for SimpleScreen CRC is determined to be the greater LoD value between the two reagent lots, which was system score (S) = 0.736821 (Lot 2).

A subsequent C95 confirmation study was conducted as part of the Precision study, consisting of unmanipulated clinical samples (12 ACN and 12 non-ACN donors, Table 3)

along with three clinical blends prepared from 2 CRC and 1 APL donor (the same unmanipulated donors were also run as part of the precision study). The study was conducted across 6 days, 3 reagent lots, 2 instrument lines and for a total of 24-36 replicates per sample. The C95 (LoD) was confirmed and re-established across the combined data from unmanipulated clinical samples and clinical blends (S = 0.711697). This confirmed C95 value was used as the C95 across all analytical validation studies.

Limit of Blank (LoB)

The Limit of Blank (LoB) of SimpleScreen CRC was evaluated by confirming that the false positive rate of the assay was 0% for samples lacking hypermethylated fragments (HMFs). Four 0% methylated contrived samples were tested across two reagent lots with 96 replicates per lot (4 samples x 24 reps per sample). Testing for each reagent lot included 3 processing runs, 4 batches per processing run, and 2 replicates per sample for each batch. The false positive rate was 0% for both reagent lots (**Table 2**).

Table 2: False Positive Rate for Two Reagent Lots

Lot	N Positive	N Total	False Positive Rate
1	0	96	0.00%
2	0	95	0.00%

B. Precision

SimpleScreen CRC precision was evaluated across two combined studies using donors selected that had a system score (S) spanning the assay range and included 8 high positives, 7 low positives, 4 borderline, 6 high negatives and 6 low negative samples.

The initial precision study evaluated 24 individual clinical donors (12 ACN and 12 non-ACN) with ACN status confirmed by colonoscopy/pathology. The ACN donors included 9 CRC and 3 APLs. The non-ACN donors included 5 colonoscopy confirmed negatives (NEG) and 7 donors with non-advanced precancerous lesions (NAPLs). Repeated measures for each donor were taken across 12 batches that were processed across 6 different days, 3 reagent lots, 2 instrument lines, and up to 2 runs per condition. Donors were tested with 2-3 replicates per batch for a total of 24-36 replicates across the 12 batches.

A supplemental precision study was performed with 7 individual clinical donors with ACN status confirmed by colonoscopy/pathology. The ACN donors included 2 CRC and 5 APLs. Repeated measures for each donor were taken across 8 batches that were

processed across 3 timepoints, 1 reagent lot and 1 instrument line. Donors were tested with 3 replicates per batch for a total of 24 replicates across the 8 batches.

To evaluate the qualitative output from the SimpleScreen CRC AI/ML-enabled classification model, the majority call for each donor was reported and individual replicates were compared to the majority call for each donor. The Correct Call Percent . for each donor is provided in **Table 3**. The majority call agreement for ACN donors ranged from 52.2% - 100% and the aggregate majority call agreement across all non-borderline ACN donors was 92.2%. The majority call agreement for non-ACN donors ranged from 86.1% - 100% and the aggregate majority call agreement across all non-borderline non-ACN donors was 94.3%.

Table 3: Detection Rates for Clinical Donors

Sample ID	System Score Bin	Colonoscopy Status	Majority Call	Mean System Score	Total Replicates	% Called Positive Test Result	% Called Negative Test Result
CD23	Low Negative	NEG	Negative	0.395289	36	0/36 (0%)	36/36 (100%)
CD10	Low Negative	NAPL	Negative	0.396721	36	0/36 (0%)	36/36 (100%)
CD19	Low Negative	NAPL	Negative	0.397109	36	1/36 (2.78%)	35/36 (97.22%)
CD11	Low Negative	NEG	Negative	0.398026	34	1/34 (2.94%)	33/34 (97.06%)
CD20	Low Negative	NEG	Negative	0.4024	36	1/36 (2.78%)	35/36 (97.22%)
CD18	Low Negative	NAPL	Negative	0.402839	36	2/36 (5.56%)	34/36 (94.44%)
CD12	High Negative	NEG	Negative	0.405771	34	1/34 (2.94%)	33/34 (97.06%)
CD2	High Negative	NAPL	Negative	0.408844	36	4/36 (11.11%)	32/36 (88.89%)
CD3	High Negative	NAPL	Negative	0.410831	36	4/36 (11.11%)	32/36 (88.89%)
CD6	High Negative	NAPL	Negative	0.410973	36	5/36 (13.89%)	31/36 (86.11%)
CD24	High Negative	NEG	Negative	0.411744	36	3/36 (8.33%)	33/36 (91.67%)
CD9	High Negative	NAPL	Negative	0.412533	35	2/35 (5.71%)	33/35 (94.29%)
CD8	Borderline	APL	Negative	0.425219	35	7/35 (20%)	28/35 (80%)
CD5	Borderline	APL	Negative	0.435738	33	8/33 (24.24%)	25/33 (75.76%)
CD1	Borderline	CRC	Negative	0.44109	23*	7/23 (30.43%)	16/23 (69.57%)

Sample ID	System Score Bin	Colonoscopy Status	Majority Call	Mean System Score	Total Replicates	% Called Positive Test Result	% Called Negative Test Result
CD29	Borderline	CRC	Positive	0.463669	24	12/24 (50%)	12/24 (50%)
CD32	Low Positive	APL	Positive	0.49472	23**	12/23 (52.17%)	11/23 (47.83%)
CD31	Low Positive	APL	Positive	0.556103	24	22/24 (91.67%)	2/24 (8.33%)
CD13	Low Positive	APL	Positive	0.576681	36	27/36 (75%)	9/36 (25%)
CD28	Low Positive	APL	Positive	0.579151	24	18/24 (75%)	6/24 (25%)
CD33	Low Positive	APL	Positive	0.637812	24	21/24 (87.5%)	3/24 (12.5%)
CD22	Low Positive	CRC	Positive	0.677421	36	31/36 (86.11%)	5/36 (13.89%)
CD30	Low Positive	APL	Positive	0.703253	24	24/24 (100%)	0/24 (0%)
CD34	High Positive	CRC	Positive	0.887552	24	24/24 (100%)	0/24 (0%)
CD4	High Positive	CRC	Positive	0.98949	35	35/35 (100%)	0/35 (0%)
CD16	High Positive	CRC	Positive	0.991532	34	34/34 (100%)	0/34 (0%)
CD14	High Positive	CRC	Positive	0.999227	36	36/36 (100%)	0/36 (0%)
CD7	High Positive	CRC	Positive	0.999525	36	36/36 (100%)	0/36 (0%)
CD21	High Positive	CRC	Positive	1	36	36/36 (100%)	0/36 (0%)
CD15	High Positive	CRC	Positive	1	35	35/35 (100%)	0/35 (0%)
CD17	High Positive	CRC	Positive	1	36	36/36 (100%)	0/36 (0%)

*One replicate failed the in-process QC metric "Library Concentration (ng/μL).
**One replicate failed the in-process QC metric "CHH Conversion Rate (%).

A variance component analysis was conducted on the system score (S) generated by the SimpleScreen CRC AI/ML enabled classification model. The system score mean, standard deviation (SD), and coefficient of variation (%CV) were calculated for each donor (**Table 4**). The total variability (within-laboratory) ranged from 0% CV to 27% CV.

Table 4 - Variance Component Estimates for the SimpleScreen CRC System Score for each Clinical Donor. Results are presented as: standard deviation (coefficient of variation)

Donor ID	ACN Status	Majority Call	Mean S	Repeatability	Within-Laboratory	Between-Day	Between-Lot	Between-Instrument	Between-Run
CD21	ACN	Positive	1	0 (0%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD17	ACN	Positive	1	0 (0%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD15	ACN	Positive	1	0 (0%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD7	ACN	Positive	0.999525	0.000842 (0.08%)	0.000862 (0.09%)	0(0%)	0.000183 (0.02%)	0 (0%)	0 (0%)
CD14	ACN	Positive	0.999227	0.001347 (0.13%)	0.001362 (0.14%)	0(0%)	0 (0%)	0 (0%)	0.000199 (0.02%)
CD16	ACN	Positive	0.991532	0.014968 (1.51%)	0.015416 (1.55%)	0(0%)	0 (0%)	0.002014 (0.2%)	0.003091 (0.31%)
CD4	ACN	Positive	0.98949	0.032519 (3.29%)	0.032604 (3.29%)	0(0%)	0 (0%)	0 (0%)	0.002344 (0.24%)
CD22	ACN	Positive	0.677421	0.178979 (26.42%)	0.178979 (26.42%)	0.000004(0%)	0 (0%)	0.000004 (0%)	0 (0%)
CD13	ACN	Positive	0.576681	0.155874 (27.03%)	0.155874 (27.03%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD1	ACN	Negative	0.44109	0.077757 (17.63%)	0.077757 (17.63%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD5	ACN	Negative	0.435738	0.052502 (12.05%)	0.055782 (12.8%)	0(0%)	0.011989 (2.75%)	0.014539 (3.34%)	0 (0%)
CD8	ACN	Negative	0.425219	0.051409 (12.09%)	0.062409 (14.68%)	0(0%)	0.000001 (0%)	0.000001 (0%)	0.035382 (8.32%)
CD9	non-ACN	Negative	0.412533	0.037887 (9.18%)	0.037887 (9.18%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD24	non-ACN	Negative	0.411744	0.04148 (10.07%)	0.04148 (10.07%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD6	non-ACN	Negative	0.410973	0.040975 (9.97%)	0.041849 (10.18%)	0.008508(2.07%)	0 (0%)	0 (0%)	0 (0%)
CD3	non-ACN	Negative	0.410831	0.040728 (9.91%)	0.040728 (9.91%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD2	non-ACN	Negative	0.408844	0.029132 (7.13%)	0.039957 (9.77%)	0(0%)	0 (0%)	0.023109 (5.65%)	0.014624 (3.58%)
CD12	non-ACN	Negative	0.405771	0.027024 (6.66%)	0.027335 (6.74%)	0(0%)	0.004117 (1.01%)	0 (0%)	0 (0%)
CD18	non-ACN	Negative	0.402839	0.021657 (5.38%)	0.022442 (5.57%)	0.005881(1.46%)	0 (0%)	0 (0%)	0 (0%)

Donor ID	ACN Status	Majority Call	Mean S	Repeatability	Within-Laboratory	Between-Day	Between-Lot	Between-Instrument	Between-Run
CD20	non-ACN	Negative	0.4024	0.014064 (3.5%)	0.014343 (3.56%)	0.002811(0.7 %)	0 (0%)	0 (0%)	0 (0%)
CD11	non-ACN	Negative	0.398026	0.010401 (2.61%)	0.011607 (2.92%)	0.002076(0.52%)	0 (0%)	0.004715 (1.18%)	0 (0%)
CD19	non-ACN	Negative	0.397109	0.015312 (3.86%)	0.015312 (3.86%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD10	non-ACN	Negative	0.396721	0.009417 (2.37%)	0.009417 (2.37%)	0(0%)	0 (0%)	0 (0%)	0 (0%)
CD23	non-ACN	Negative	0.395289	0.007055 (1.78%)	0.008578 (2.17%)	0.00488(1.23 %)	0 (0%)	0 (0%)	0 (0%)

C. Analytical Specificity

In-silico Analysis of Primers and Probes

The specificity of the primers used in SimpleScreen CRC was evaluated by testing the potential of the primers to amplify the non-specific products from human DNA or from the genomes of commensal microorganisms using publicly available sequences in an in-silico analysis. Study results found no potential for meaningful off-target amplification.

The probe specificity was evaluated by mapping probe sequences to possible contaminant non-human sequences. Study results found no potential for meaningful binding to enterobacterial or viral non-specific targets (common bacterial, fungal, and viral sources that are commonly found in the human blood or on the skin).

D. Non-CRC Cancer and Comorbid Conditions Cross Reactivity

To evaluate the cross-reactivity of SimpleScreen CRC, non-CRC cancers and non-tumor chronic conditions that may share analytes with CRC and APLs were assessed and the positivity rate in non-CRC conditions when tested with SimpleScreen CRC was established.

Whole blood samples collected from subjects with non-CRC cancers and non-cancer conditions were selected and processed using the SimpleScreen CRC workflow to generate a classification call , which was used to assess cross-reactivity. These samples were collected from subjects with a known diagnosis of cancer or disease, which are not representative of an asymptomatic intended use population. The result is an overestimation of the non-CRC cancer or disease detection. Sample types and sizes from subjects with the conditions of interest, listed below in **Table 5,** were the target of this study.

Table 5: Non-CRC Cancer and Non-Cancer Conditions Cross Reactivity Sample Size

Sample Type	Sample Type	Sample Size
Non-CRC Cancers	Prostate Cancer	27
	Breast Cancer	27
	Lung Cancer	26
	Pancreatic Cancer	26
	Kidney Cancer	7
	Bladder Cancer	9
	Stomach Cancer	5
	Liver Cancer	11
Non-Cancer Conditions	Rheumatoid Arthritis	244
	Chronic Obstructive Pulmonary Disease	154
	Diabetes Type II	1442
	Inflammatory Bowel Disease	27
	Chronic Kidney Disease	117
	Systemic Lupus Erythematosus	52
	Hypertension	7769
	Dyslipidemia	3392
	Diverticulitis	98
	Diverticulosis	169
	Chronic Gastritis	105
	Esophagitis	46
	Chronic Liver Disease	229
	Coronary Heart Disease/Coronary Artery Disease/Other Cardiovascular Disease*	336

*Other cardiovascular diseases (CVDs) categories are based on CVD prevalence (conditions involved in heart or blood vessels such as myocardial infarction, peripheral artery disease, etc.) and based on patient population data.

Using an established analytical approach, the positive detection fraction was calculated as the number of SimpleScreen CRC positive samples out of the total number of passing samples tested per condition. The cross-reactivity/positivity rates in non-CRC cancers and non-cancer conditions when tested with SimpleScreen CRC are listed in **Table 6** and **Table 7**, respectively.

Table 6: Cross-reactivity (Positivity) Rate for Non-CRC Cancers

Cancer	Percent Positive Call n/N (%)	Annual Incidence rate per 10000 (45-84)*	Number of SimpleScreen CRC positive calls per 10000§
Prostate Cancer†	7/27 (25.9%)	18.63	4.8
Breast Cancer	9/27 (33.3%)	17.48	5.8

Cancer	Percent Positive Call n/N (%)	Annual Incidence rate per 10000 (45-84)*	Number of SimpleScreen CRC positive calls per 10000§
Lung Cancer	16/26 (61.5%)	13.83	8.5
Pancreatic Cancer	18/26 (69.2%)	3.72	2.6
Kidney Cancer	0/7 (0.0%)	4.77	0
Bladder Cancer	3/9 (33.3%)	4.81	1.6
Stomach Cancer	4/5 (80.0%)	1.89	1.5
Liver Cancer	11/11 (100.0%)	2.84	2.8

* Information was obtained from National Cancer Institute (http://seer.cancer.gov; last accessed 09 July, 2025)
† Value adjusted to represent sex-specific disease state.
§ Calculated by multiplying positive call rates with annual incidence.
CRC = colorectal cancer

Table 7: Cross-reactivity (Positivity) Rate for Non-Cancer Conditions

Disease	Percent Positive Call n/N (%)	Prevalence Observed in PREEMPT CRC*	Number of SimpleScreen CRC positive calls per 10000§
Rheumatoid Arthritis	24/244 (9.8%)	0.9%	8.8
Chronic Obstructive Pulmonary Disease	19/154 (12.3%)	0.9%	11.1
Diabetes Type II	176/1442 (12.2%)	5.9%	72.0
Inflammatory Bowel Disease† ^	4/27 (14.8%)	1.3%	19.2
Chronic Kidney Disease	12/117 (10.3%)	0.5%	5.2
Systemic Lupus Erythematosus	2/52 (3.8%)	0.2%	0.8
Hypertension	739/7769 (9.5%)	29.5%	280.3
Dyslipidemia	303/3392 (8.9%)	12.6%	112.1
Diverticulitis	11/98 (11.2%)	0.5%	5.6
Diverticulosis	16/169 (9.5%)	0.6%	5.7
Chronic Gastritis	4/105 (3.8%)	0.4%	1.5
Esophagitis	2/46 (4.3%)	0.2%	0.9
Chronic Liver Disease	29/229 (12.7%)	0.9%	11.4
Coronary Heart Disease/Coronary Artery Disease/Other Cardiovascular Disease	44/336 (13.1%)	1.4%	18.3

* Prevalence of non-cancer conditions was observed from PREEMPT CRC all enrolled subjects (N=48,995) with the exception of IBD.
† Data obtained from Dahlhamer et al.[1]
^Individuals with diagnosed IBD are contraindicated for SimpleScreen CRC. Prevalence estimates for undiagnosed IBD could not be determined from PREEMPT CRC.
§Calculated by multiplying positive call rates with PREEMPT CRC observed prevalence and 10000.

E. Carryover and Cross-contamination

To evaluate carryover and cross-contamination effects on the SimpleScreen CRC, alternating low and high analyte samples were processed in a checkerboard layout in 6 batches, 92 samples per batch plus controls, performed consecutively on a single line of instruments and a single side of the sequencer. A total of 273 unmethylated and 279 methylated contrived sample replicates were used in this study. Of the methylated contrived samples, 279/279 (100.0%) produced valid positive results. Of the unmethylated contrived samples, 269/272 (98.8%) produced valid negative results. The three false positive results all had a DNA input of 20ng and system score below the C95 (Limit of Detection).

F. Interfering Substances

Whole blood contains endogenous substances that may interfere with an assay when present at elevated levels. To evaluate the impact of potential endogenous and exogenous interfering substances on the performance of SimpleScreen CRC, 24 replicates each of clinical blend and healthy multi-donor pool plasma samples, which simulate CRC-negative samples, were tested in the absence (control) and presence (test) of 9 endogenous substances (**Table 8**) and 4 exogenous substances (gDNA, magnetic beads, ethanol, and molecular index barcodes). The difference in system score drift was 0.4% - 5.1%, which was considered acceptable. No interference on the performance of SimpleScreen CRC test was observed for any of the substances at the concentration tested.

Table 8: Endogenous Interfering Substances Tested

Interfering Substance	Level
Bilirubin, unconjugated	40 mg/dL
Bilirubin, conjugated	40 mg/dL
Hemoglobin (hemolysate)*	1,000 mg/dL
Triglycerides	1,500 mg/dL
Albumin	6 g/dL
Cholesterol	400 mg/dL
Red Blood Cells	0.4% v/v
Uric acid	23.5 mg/dL
Glucose	1,000 mg/dL

*Hemoglobin and Hemolysate are used interchangeably

G. Robustness

Assay Workflow Guardbanding

The objective of the guardbanding study was to evaluate the tolerance of the SimpleScreen CRC test to variations in critical assay workflow parameters such as the incubation temperatures, reagent volumes, reagent concentrations, throughout the entire

SimpleScreen CRC workflow. Guardbanding for the SimpleScreen CRC test was performed using a risk-based approach to identify assay components for guardbanding and their respective ranges (guardbands).

Three sample types, contrived high methylated, contrived low methylated, and healthy donor pooled plasma were used for testing 43 factors across the SimpleScreen CRC test from extraction to target enrichment.

There was no impact to the system score (S) and the final classification results due to the guardbanded design factors indicating the SimpleScreen CRC classification is robust with regard to the guardbanded factors. Across all guardbanded conditions, contrived high samples demonstrated 100% majority call agreement, contrived low samples maintained 81.0-91.3% majority call agreement, and healthy donor plasma reported a majority call agreement of 91.7-100.0%.

DNA Input Mass

To evaluate the performance of SimpleScreen CRC at the predicted range of cfDNA input mass of the Intended Use Population (IUP), 4 cfDNA blends, at 3 cfDNA levels (3.5 ng, 11 ng, and 70 ng), at a minimum of 20 replicates per sample per cfDNA input mass level (288 replicates total) were tested over 4 batches. The study acceptance criteria was met, demonstrating that the SimpleScreen CRC test produces a 94.5% (75/79, 95% CI: 87.5%-98.6%) positivity rate qualitative output at the minimum and a 100% (44/44, 95% CI: 92.0%-100.0%) at the maximum limits of the predicted 95% IUP distribution (3.5 ng and 70 ng, respectively) comparable to the standard input mass of 11 ng.

H. Stability

Whole Blood Stability

Whole blood stability was assessed using whole blood samples collected in Streck cfDNA BCTs from 25 self-identified healthy donors from the IUP, 9 APL donors, and 35 CRC donors. Blood samples were stored at room temperature (16°C – 25°) for up to seven days before plasma separation and downstream processing with SimpleScreen CRC. From each donor, blood was collected in BCTs from two to three lots with various ages from the date of sterilization (DOS) or date of manufacture (DOM) of the Streck cfDNA BCTs (Lot A: ≤ 30 days from DOS at study initiation, Lot B: 12-18 months from DOM, Lot C: > 18 months from DOM).

From each self-identified healthy donor, 18 BCTs were collected with six BCTs from each of the three lots. From the CRC donors and APL donors, four BCTs were collected. CRC donors and APL donors were only collected with either Lot A and Lot C or Lot A and Lot B due to the limited number of BCTs that could be collected from these donor types.

For each lot, half of the BCTs from each donor were processed to plasma on the day of receipt at Freenome (Day 1 post blood draw) and the other half were stored for seven days before being processed to plasma to support stability of whole blood specimens collected in BCTs for up to seven days.). NPA and PPA were evaluated by comparing binary SimpleScreen CRC results from Day 8 to the Day 1 majority call across lots .

The study results support that whole blood samples are stable when stored at room temperature (16°C – 25°) for up to seven days when collected in Streck cfDNA BCTs aged up to 18 months past the DOM for use in conjunction with SimpleScreen CRC.

An additional stability study was performed to demonstrate that the device performance for whole blood processed on Day 1 post draw can represent the device performance for samples processed on the date of collection. Whole blood was collected in four BCTs from each of 32 unique donors (20 healthy and 12 CRC donors). For each donor, 2 BCTs were processed to plasma within 4 hours and 2 BCTs were processed to plasma on Day 1. NPA and PPA were evaluated by comparing binary SimpleScreen CRC results from the test condition (Day 1) to the within 4 hour (control) majority call and a PPA of 100% and NPA of 90% were observed demonstrating whole blood processed on Day 1 post blood draw represents the device performance for samples processed on the data of collection.

Whole Blood Transportation Stability

Whole blood stability across different shipping conditions was assessed using whole blood samples collected in Streck cfDNA BCTs from 21 self-identified healthy donors, 18 colonoscopy-confirmed negative donors, 1 non-advanced adenoma (NAA) donor, 7 APL donors, and 15 CRC donors.

Whole blood samples were either processed to plasma on the day of receipt (Day 1 post-collection, control condition) or packaged in the SimpleScreen CRC blood collection kit and exposed to winter or summer shipping conditions. Samples were then stored at room temperature (15°C – 25°C) until either Day 5 or Day 8 post-collection when plasma was isolated.

A total of seven study conditions (BCTs stored inverted Summer Day 8, upright Summer Day 8, inverted Winter Day 8, upright Winter Day 8, upright Summer Day 5, upright Winter Day 5, and Day 1 control). From each self-identified healthy donor, blood was collected into 21 BCTs with three replicates (i.e., three BCTs) for each of the seven conditions. For ACN and colonoscopy-confirmed negative/NAA donors, blood was collected into five BCTs with one BCT for each of the four conditions where plasma was isolated on Day 8 post-collection in addition to the control. The PPA was 100% for all conditions (Summer/Winter, Inverted/Upright) tested and the NPA for Winter Upright, Summer Upright, Winter Inverted, and Summer Inverted, was 95.8%, 95.7%, 93.9%, and 91.8%, respectively

Plasma Stability: Freeze-Thaw

A freeze-thaw plasma stability study was conducted to evaluate the impact of plasma freeze-thaws on the performance of the SimpleScreen CRC assay. A total of 20 healthy, 10 CRC, and 4 APL samples were tested across five freeze-thaw cycles. No notable degradation in the system score in the stability study was observed over at the fifth freeze-thaw demonstrating plasma samples are stable for up to four freeze-thaw cycles.

Plasma Stability: Long-term

A long-term plasma stability study was designed to evaluate the stability of plasma under long-term storage conditions at -80°C (-70°C - -90°C). A total of 18 healthy, 15 CRC and 13 APL samples to be tested across 5 time-points. This study is ongoing, and results were obtained for the T0, T1 (12 months), and T2 (24 months) time-points. No notable degradation in the system score the stability study was observed at 24 months. Currently, this study has demonstrated stability for at least 22 months when stored at -80ºC.

Plasma Stability: On-board

An on-board plasma stability study was conducted to evaluate the stability of plasma when stored up to eight hours at room temperature (16°C – 25°C) prior to plasma plating. Two control batches were thawed and then proceeded immediately to plasma plating and two test condition batches were held at room temperature for a total of nine hours. Each batch contained 10 replicates for each of two clinical blend samples as well as 20 replicates for one healthy multi-donor pool (HMDP), resulting in a total of 40 replicates for clinical blends and 40 replicates for HMDP for both the control and test condition. The percent difference in system scores between the control and treatment conditions was less than 2% for both the clinical blends and the HMDP. The PPA for both clinical blends was 100.0% for both the treatment and control condition. The NPA for the HMDP was 87.5% in the treatment condition and 95.0% in the control condition.

cfDNA Stability
cfDNA stability was evaluated with a total of 18 clinical blended samples (3 clinical blends x 6 hypermethylated fragment [HMF] levels), 4 unmethylated (0% methylated) contrived samples from manufacturing events, and batch controls (PC, NC, and NTC) at each timepoint (e.g., baseline, 6.5 months, 16 months, and 19 months). No significant differences were observed between baseline and later timepoints, demonstrating cfDNA stability for 16 months when stored at -20°C.

In-Use Stability
An in-use stability study was conducted to evaluate the stability of 4 hold-points in the SimpleScreen CRC workflow. The in-use stability study utilized 2 clinical blend samples and one healthy multi-donor pool plasma sample. Twenty clinical blend replicates were tested per batch per condition (10 replicates per clinical blend) and 20 healthy multi-donor pool replicates tested per batch per condition. One control batch and one test batch was run per condition. The percent change in mean S was compared between the control and the test condition to verify the following in-use stability:

- Samples can be stored after cfDNA extraction at -20°C for 34 days
- Samples can be stored after library preparation at -20°C for 37 days
- Samples can be stored after target capture and DNA quantification at -20°C for 32 days
- Samples can be stored after normalization and pooling at -20°C for 35 days

Test condition samples were tested at the conditions listed above and compared against the control (baseline) samples. The in-use stability study demonstrated a PPA of 100% and NPA of ≥90% for all test conditions listed and the study met all acceptance criteria. There was no notable difference in mean system score S between the control and test samples for the 4 tested hold conditions, supporting the use of the hold points as part of the assay process.

Reagent Shelf-Life Stability
A real-time reagent stability study was conducted to establish the shelf-life of SimpleScreen CRC reagents and external batch controls. Three SimpleScreen CRC lots were tested at six timepoints (T0: after QC release; T1: 2 months after T0; T2: 4 months after T0; T3: 6 months after T0; T4: 8 months after T0; T5: 10 months after T0) with 3 unique clinical blend samples at approximately 1.5 times the C95, 3 unique clinical blend

samples at approximately 3 times the C95, and three healthy multi-donor pool plasma samples. A minimum of 24 replicates per sample type per reagent lot per timepoint were tested. The study acceptance criteria were met and verified the shelf-life stability of SimpleScreen CRC reagents and external batch controls to be a minimum of 10 months when stored at the appropriate conditions.

On Board Reagent Stability

The on-board stability study evaluated the stability for SimpleScreen CRC reagents while on deck (stored on the relevant instrument) of their respective automation platforms and while off deck (stored separate to instrument, e.g. on the bench) for the genomics portion of the SimpleScreen CRC test workflow:

- cfDNA extraction kit
- Library preparation kit
- DNA quantification kit
- Next generation sequencing kit and manufacturer-provided sequencer control

The study consisted of three batches which tested most challenging time and temperature combinations. Across the three batches, a total of 60 clinical blend samples, 60 healthy multi-donor pool samples at their native system score, and three of each batch control were tested. A total of 20 replicates per condition were tested to evaluate the tolerance of the SimpleScreen CRC assay to the final hold conditions tested. The results demonstrate the reagents are stable for the amount of time that they are stored in use onboard the instrument.

Output Plate Freeze-Thaw Stability

An output plate freeze-thaw stability study was conducted to evaluate the freeze-thaw stability of the 7 of 9 safe stopping points in the SimpleScreen CRC workflow at which intermediate samples are stored frozen as well as one on-board stability condition (extraction plate on-board stability). The study utilized two clinical blend samples and one HMDP sample. Twenty clinical blend replicates were tested per batch per condition (10 replicates per clinical blend) and 20 healthy multi-donor pool replicates tested per batch per condition. One control batch and two test batches were evaluated to assess the maximum number of freeze-thaw events and the on-board stability of the extraction output plates. The study met all acceptance criteria. The percent change in the mean system score between the control and test conditions was evaluated, and the percent drift in the mean score for the test condition remained within the acceptable range. Output plate freeze-thaw stability demonstrated an overall PPA of 100% and NPA of 90%. The SimpleScreen intermediate samples stored at the evaluated safe stopping

points are stable for up to three freeze-thaw cycles. In addition, the extraction output plate is stable for up to 2.5 hrs when stored on-board at room temperature.

Reagent Freeze-Thaw Stability

A reagent freeze-thaw stability study was conducted to evaluate the impact of freeze-thaw cycles on reagents used in SimpleScreen CRC that are stored at -20°C. In addition, the study evaluated the freeze-thaw stability of intermediate reagents (aged master mixes). The study tested two clinical blends (10 replicates each) and one HMDP(20 replicates) per batch under each test condition. One control batch and two test batches were evaluated to determine the maximum number of freeze-thaw cycles that could be tolerated without affecting assay performance. The percent change in the mean system score between the control and test conditions was evaluated, and overall the percent drift in the mean score for the test condition remained within the acceptable range (<10%). The reagent freeze-thaw stability study demonstrated an overall PPA of 95% and NPA of 90% under the most challenging test condition. The study met all acceptance criteria. The SimpleScreen CRC reagents, master mixes, and sequencing reagents are stable for up to four, three, and three freeze-thaw cycles, respectively.

I. Reagent Lot Interchangeability

The Reagent Lot Interchangeability study was conducted to evaluate the interchangeability of nine critical reagent groups across three reagent lots using 24 clinical blend replicates (eight replicates per three clinical blends) and 24 HMDP replicates (eight replicates per three HMDP samples). Reagent lot interchangeability for the seven reagent group was demonstrated by an overall PPA of 100% and an overall NPA of 92% across nine interchangeability conditions. The remaining two groups demonstrated 100% pass rate and 100% concordance with the reference condition. In addition, the overall percent drift in the mean system score for the interchangeability conditions remained within the acceptable range (<10%) for both clinical blends and healthy multi-donor pools (HMDPs). All acceptance criteria were met.

J. Blood Collection Tube Characterization and Concordance Studies

Streck cfDNA BCT Lot-to-lot Reproducibility Study

A lol-to-lot reproducibility study was conducted to evaluate performance of the Streck cfDNA BCT across lots when used with SimpleScreen CRC. Lot-to-lot reproducibility was evaluated using whole blood samples collected across five BCTs, each from a different lot, from 21 healthy donors and 16 ACN donors from the IUP. The PPA and NPA were evaluated by comparing the binary SimpleScreen CRC results for each lot to the majority call across lots. A PPA and NPA of 100% were observed.

Streck cfDNA BCT Tube-to-tube Repeatability Study

A tube-to-tube repeatability study was conducted to evaluate performance between Streck cfDNA BCTs when used with SimpleScreen CRC. Tube-to-tube repeatability was evaluated using whole blood samples collected across five BCTs, each from the same lot, from 20 healthy donors and 19 ACN donors from the IUP. The PPA and NPA were evaluated by comparing the binary SimpleScreen CRC results for each BCT to the majority call across all BTCs. A PPA and NPA of 100% were observed.

Streck cfDNA BCT Preservative Guardbanding Study

This study was conducted to evaluate the performance of SimpleScreen CRC in the presence of excess components from Streck cfDNA BCT preservatives. Samples were collected into the standard Streck cfDNA BCT preservative condition and four excess preservatives conditions for Streck cfDNA BCTs (**Table 9**).

Table 9 - Study Design Overview

Condition (BCT Type Description)	Streck cfDNA BCT Preservative		
	Component A Concentration (relative to standard)	Component B Concentration (relative to standard)	Preservative Volume (relative to standard)
A (Standard)	1X	1X	1X
B (2X Component A)	2X	1X	1X
C (2X Component B)	1X	2X	1X
D (8 mL short draw)	1X	1X	1.25X
E (5 mL short draw)	1X	1X	2X

From each self-identified healthy donor, 15 BCTs were collected with three replicates (i.e., three BCTs) from each condition. From the APL donors, five BCTs were collected with one BCT for each condition. From CRC donors, three BCTs were collected with one BCT for conditions A, B, and C or for conditions A, D, and E due to the limited number of BCTs that could be collected.

21 healthy and 20 ACN donors were included in the evaluation of condition B relative to the control. 20 healthy and 20 ACN donors were included in the evaluation of condition C relative to the control. 21 healthy donors and 17 ACN donors were included in the evaluation of conditions D and E relative to the control.

The NPA and PPA were evaluated by comparing the binary SimpleScreen CRC results from the test conditions to the control condition majority call. The result of the guardbanding study showed underfilling the BCTs with less than 10 mL of blood may impact the SimpleScreen CRC test results (**Table 10)**.

Table 10 - Streck Preservative Guardbanding PPA and NPA Results

Health Status	Metric	Sample Bin	Condition	Point Estimate
Healthy	NPA	<C5	Control	100.0%
			8 mL Short Draw	91.4%
			5 mL Short Draw	86.1%
			2X Component A Interference	91.4%
			2X Component B Interference	97.2%
	NPA	C5 - C95	Control	83.3%
			8 mL Short Draw	91.7%
			5 mL Short Draw	91.7%
			2X Component A Interference	91.7%
			2X Component B Interference	90.9%
ACN+	NPA	<C5	Control	100.0%
			8 mL Short Draw	50.0%
			5 mL Short Draw	66.7%
			2X Component A Interference	66.7%
			2X Component B Interference	77.8%
	NPA	C5 - C95	Control	100.0%
			8 mL Short Draw	100.0%
			5 mL Short Draw	100.0%
	PPA		Control	100.0%

			8 mL Short Draw	100.0%
			5 mL Short Draw	50.0%
			2X Component A Interference	100.0%
			2X Component B Interference	0.0%
	PPA	>C95	Control	100.0%
			8 mL Short Draw	87.5%
			5 mL Short Draw	87.5%
			2X Component A Interference	90.0%
			2X Component B Interference	90.0%

Streck cfDNA BCT Incomplete and Overmixing Verification Study
This study was conducted to evaluate the robustness of SimpleScreen CRC to incomplete or over mixing of blood with the anticoagulant and cell preservative in Streck cfDNA BCTs. Samples were collected in BCTs and subject to three study conditions, standard mixing (10 inversions), incomplete mixing (5 inversions) and over mixing (15 inversions). 21 healthy donors with three replicates (i.e., three BCTs) for each condition and 16 ACN (CRC or APL) donors with one replicate (i.e., one BCT) for each condition were included in the evaluation.

Bias in system score relative to the control was evaluated. Although statistically significant positive bias in the incomplete mixing condition was observed, the observed bias for both test conditions fell within the acceptable range NPA and PPA were evaluated by comparing binary SimpleScreen CRC results from the test conditions to the control condition majority call. The results indicated that incomplete mixing may result in diminished performance.

Pivotal Clinical Study

The Prevention of Colorectal Cancer Through Multiomics Blood Testing (PREEMPT CRC) study[2] was a prospective, multi-center study conducted to generate data to support the safety and effectiveness of SimpleScreen CRC as a blood-based screening test for the detection of markers associated with the presence of CRC from whole blood samples. To evaluate the performance of the SimpleScreen CRC blood test, the test

result (negative or positive) was compared with the histopathological results of the index lesion (most clinically significant lesion) identified from screening colonoscopy. The pre-specified primary performance measures included sensitivity for CRC, and specificity for ACN. A secondary measure was sensitivity for APLs. Additional performance measures included positive predictive value (PPV) for CRC and APL and negative predictive value (NPV) for CRC and ACN.

A. Study Design

PREEMPT CRC (ClinicalTrials.gov Identifier: NCT04369053) was a prospective study enrolling a total of 48,995 participants at 200 sites in the United States and one site in the United Arab Emirates (at a facility affiliated with a US healthcare system). Eligible participants provided signed informed consent and were enrolled from May 19, 2020, to April 1, 2022. However, to mitigate the then unknown impact of the COVID-19 pandemic on the representativeness of the study population, the pivotal clinical validation population was limited to participants consecutively enrolled and eligible on or after April 5, 2021, to April 1, 2022, for a total of 31,594 participants. Enrolled subjects were aged 45 to 85 years within 30 days of enrollment, asymptomatic, at average risk for CRC, were willing to undergo blood collection (site-based and mobile phlebotomy), and a standard-of-care (SOC) screening colonoscopy within 90 days of blood draw. The window to complete colonoscopy was increased to 120 days to account for decreased access to care due to the COVID-19 pandemic. The blood draw was completed prior to colonoscopy bowel preparation. An enrichment target of ≥70% of participants between the age of 65-85 was planned but not achieved.

To evaluate the performance of SimpleScreen CRC, the test result was compared to the colonoscopy result and histopathological information. When multiple lesions were found, the most clinically significant finding was defined as the index lesion (**Table 11**). All colonoscopy reports and applicable histopathology reports were reviewed by a central pathologist to assign a histopathological category.

Table 11: Histopathological categories and definitions, including subtype definitions for APL, NAPL, and NEG

Subcategory	Findings
CRC 1.0	All stages (I-IV)

APL 2.1	Adenoma with carcinoma in situ or high-grade dysplasia, any size
APL 2.2	Adenoma, villous growth pattern (≥25%), any size
APL 2.3	Adenoma ≥1.0 cm in size
APL 2.4	Serrated lesion, ≥ 1.0 cm (includes sessile serrated adenoma/polyp)
APL 2.5	Traditional serrated adenoma (TSA), any size
NAPL 3.0	≥5 adenomas, all <1.0 cm in size, non-advanced
NAPL 4.0	3 to 4 adenomas, all <1.0 cm in size, non-advanced
NAPL 5.1	1 to 2 adenomas, all <1.0 cm in size, non-advanced
NAPL 5.2	Hyperplastic polyps (HP) ≥1.0 cm
NAPL 5.3	All SSL <1.0 cm, and HP <1.0 cm not in sigmoid or rectum
NEG 6.1	Negative and other findings upon histopathological review
NEG 6.2	No findings on colonoscopy, no histopathological review

All participants, site staff, and central pathologists remained blinded to the blood test results. Individuals conducting the SimpleScreen CRC laboratory testing were blinded to all clinical data.

a. <u>Clinical Inclusion and Exclusion Criteria</u>

Inclusion Criteria

For enrollment in PREEMPT CRC, the subjects met all the following criteria:

1. 45-85 years of age (inclusive) within 30 days of enrollment
2. Willing to undergo a SOC colonoscopy within 90 days of blood collection
3. Considered by a physician or healthcare provider to be average-risk for CRC
4. Able and willing to provide blood samples per protocol
5. Able to comprehend and willing to sign and date the written informed consent document(s)

Exclusion Criteria

Subjects were excluded from enrolling in PREEMPT CRC if they met any of the following criteria:

1. Family history of CRC
    1.1. At least one (1) first-degree relative diagnosed with CRC before the age of 60 (Note: First degree relatives include parents, siblings, and offspring)

1.2. At least two (2) first-degree relatives diagnosed with CRC at any age

2. Known hereditary gastrointestinal cancer syndrome including but not limited to the following:
   2.1. Hereditary non-polyposis CRC syndrome or Lynch syndrome
   2.2. Familial adenomatous polyposis (FAP)
   2.3. Cowden's syndrome
   2.4. Juvenile polyposis syndrome
   2.5. MUTYH-associated polyposis (MAP)
   2.6. Peutz-Jeghers syndrome
   2.7. Serrated polyposis syndrome
3. Personal history
   3.1. CRC or colorectal adenoma
   3.2. History of malignancy (except for non-melanomatous skin cancer) at the time of enrollment, and for the 5 years preceding enrollment
   3.3. IBD, including CUC and Crohn's disease
   3.4. Total colonic resection
   3.5. Cystic fibrosis
   3.6. Colonoscopy in the 9 years preceding enrollment with the exception of an incomplete colonoscopy, or for a poor or inadequate bowel prep
   3.7. Sigmoidoscopy or CT colonography in the 4 years preceding enrollment
   3.8. Stool DNA testing in the 2 years preceding enrollment
   3.9. Fecal occult blood testing or fecal immunochemical testing in the 6 months preceding enrollment
   3.10. Requiring an immediate or emergent colonoscopy for the investigation of symptoms
   3.11. Solid organ or bone marrow transplantation
   3.12. Blood product transfusion in the 120 days preceding enrollment
   3.13. Any trauma or surgery requiring overnight inpatient hospitalization in the 30 days preceding enrollment
4. A medical condition which, in the opinion of the investigator, should preclude enrollment into the study.
5. Participated or currently participating in a clinical research study in which an experimental medication has been administered during the 60 days leading up to and including the date of providing informed consent or may be administered through the time of the colonoscopy.
6. Known to be pregnant.

b. <u>Clinical Endpoints</u>

The primary objectives of the study were to determine the sensitivity of

SimpleScreen CRC for CRC and specificity for ACN using screening colonoscopy with histopathology, as ground truth, for assessment of the target condition, within 90 days of the blood draw.

Prespecified primary objectives and acceptance criteria are listed in **Table 12**. The secondary objective was to evaluate the test sensitivity for APL. The confidence intervals (CIs) for sensitivity and specificity were calculated throughout using the Wilson Score method.

Table 12: Clinical Study Primary and Secondary Objectives

Primary Objectives	Acceptance Criteria
CRC Sensitivity	Lower bound of the 2-sided 95% confidence interval (CI) exceeds 65.0%
ACN Specificity	Lower bound of the 2-sided 95% CI exceeds 85.0%

B. Accountability of PMA Cohort

There were 32731 subjects who enrolled after April 5, 2021. Of these subjects, 1137 did not meet eligibility criteria (773 subjects withdrew from the study, and 364 subjects did not have a valid blood sample). Subject disposition is summarized below.

- The pivotal clinical performance study included 31594 eligible subjects (subjects who met inclusion and exclusion criteria)
- Of the 31594 subjects, 2497 subjects were excluded due to loss to follow-up (182 subjects) or colonoscopy not performed (2315 subjects)
- Of the remaining 29097 subjects, 1275 subjects were excluded due to colonoscopy/histopathology issues (e.g. unevaluable colonoscopy, unconfirmed diagnosis).
- Of the remaining 27822 subjects, 812 subjects were excluded due to sample issues.

Of the 29097 subjects with a blood sample collected, 801 were excluded because the sample was not usable and/or could not be processed by the lab. Among 28296 (=29097-801) subjects, 13 had no result obtained due to an assay failure and 630 subjects required more than two BCTs to obtain a test result, which were considered to be re-draw events. The overall rate of “No Result” was calculated to be (13 + 630) / 29097 = 2.2% with 95% CI: (2.0%-2.4%).

The remaining 27010 subjects who had confirmed clinical findings and valid SimpleScreen CRC blood test results comprised the evaluable dataset. with 72 CRC, 2567 APLs, 8936 NAPLs and 15435 NEGs. This population was used for the primary analysis of the clinical validation of the SimpleScreen CRC test.

C. Study Population Demographics and Baseline Parameters

The demographic and baseline characteristics for subjects in the primary effectiveness cohort (evaluable cohort of 27,010 participants) are presented in **Table 13**. The average age was 58.1 years and 55.8% were female. Compared to the U.S. census distribution[3], there was a higher proportion of participants aged 50 to 54 years (32.9% vs 15.6%) and lower proportion of those aged 75 years and older (3.0% vs 12.5%) and a higher proportion of females (55.8% vs 51.8%). Regarding race, 8.8% were Asian, 11.2% Black or African American, 73.0% White, 0.3% Native Hawaiian or Other Pacific Islander, and 0.3% American Indian or Alaska Native. Regarding ethnicity, 11.8% were Hispanic or Latino. The mean BMI was 29.4 kg/m^2.

Consistent with disease etiology, subjects with CRC or APLs tended to be slightly older than those without any ACN (59.9y or 58.8y vs 58.0y) and male (51.4% or 55.0% versus. 43.0%).

Demographic characteristics are summarized for the evaluable cohort across histopathology categories (CRC, APLs, ACN, and no ACN) (**Table 13**).

Table 13: Demographic and Baseline Characteristics for the Primary Effectiveness Cohort

Characteristic	Evaluable Cohort (N=27010)[I,II]	CRC (N=72)[I,II]	APL (N=2567)[I,II]	ACN (N=2639)[I,II]	No ACN (N=24371)[I,II]
Age (Years)[III]					
n	27010	72	2567	2639	24371
Mean (SD)	58.1 (8.2)	59.9 (9.0)	58.8 (8.2)	58.8 (8.2)	58.0 (8.2)
Median	57.0	59.0	59.0	59.0	57.0
Min, Max[IV]	45.0, 86.0	46.0, 82.0	45.0, 84.0	45.0, 84.0	45.0, 86.0
Age Category (Years), n (%)					
[45 to 49]	2968 (11.0%)	7 (9.7%)	247 (9.6%)	254 (9.6%)	2714 (11.1%)
[50 to 54]	8899 (32.9%)	18 (25.0%)	769 (30.0%)	787 (29.8%)	8112 (33.3%)
[55 to 64]	8725 (32.3%)	24 (33.3%)	885 (34.5%)	909 (34.4%)	7816 (32.1%)
[65 to 74]	5604 (20.7%)	19 (26.4%)	587 (22.9%)	606 (23.0%)	4998 (20.5%)

Characteristic	Evaluable Cohort (N=27010)[I,II]	CRC (N=72)[I,II]	APL (N=2567)[I,II]	ACN (N=2639)[I,II]	No ACN (N=24371)[I,II]
[75 and greater]	814 (3.0%)	4 (5.6%)	79 (3.1%)	83 (3.1%)	731 (3.0%)
Sex, n (%)					
Male	11934 (44.2%)	37 (51.4%)	1413 (55.0%)	1450 (54.9%)	10484 (43.0%)
Female	15076 (55.8%)	35 (48.6%)	1154 (45.0%)	1189 (45.1%)	13887 (57.0%)
Ethnicity, n (%)					
Hispanic or Latino	3189 (11.8%)	9 (12.5%)	221 (8.6%)	230 (8.7%)	2959 (12.1%)
Not Hispanic or Latino	22421 (83.0%)	62 (86.1%)	2208 (86.0%)	2270 (86.0%)	20151 (82.7%)
Unknown	1400 (5.2%)	1 (1.4%)	138 (5.4%)	139 (5.3%)	1261 (5.2%)
Race, n (%)					
American Indian or Alaska Native	78 (0.3%)	0 (0.0%)	9 (0.4%)	9 (0.3%)	69 (0.3%)
Asian	2381 (8.8%)	14 (19.4%)	132 (5.1%)	146 (5.5%)	2235 (9.2%)
Black or African American	3038 (11.2%)	7 (9.7%)	251 (9.8%)	258 (9.8%)	2780 (11.4%)
Native Hawaiian or Other Pacific Islander	72 (0.3%)	0 (0.0%)	11 (0.4%)	11 (0.4%)	61 (0.3%)
White	19707 (73.0%)	48 (66.7%)	2023 (78.8%)	2071 (78.5%)	17636 (72.4%)
Other	543 (2.0%)	2 (2.8%)	34 (1.3%)	36 (1.4%)	507 (2.1%)
Unknown	1055 (3.9%)	1 (1.4%)	87 (3.4%)	88 (3.3%)	967 (4.0%)
More Than One Race	136 (0.5%)	0 (0.0%)	20 (0.8%)	20 (0.8%)	116 (0.5%)
BMI (kg/m^2)					
n	26943	72	2561	2633	24310
Mean (SD)	29.4 (6.4)	30.6 (7.1)	30.6 (6.7)	30.6 (6.7)	29.2 (6.4)
Median	28.3	29.1	29.5	29.5	28.2
Min, Max	10.8, 78.6	18.7, 50.0	15.6, 75.5	15.6, 75.5	10.8, 78.6
Missing	67	0	6	6	61

[I] Percentage based on N in column heading.
[II] Columns are not mutually exclusive.
[III] Age was recorded based on year of birth; fractional age was not calculated.
[IV] Per eligibility, the study population included subjects aged 45 to 85, within one month of enrollment. Subjects outside this age range were confirmed by the respective sites to meet the age eligibility at time of enrollment.
BMI = body mass index; SD = standard deviation

D. Safety and Effectiveness Results

a. Safety Results

There were no device-related serious adverse events (SAEs) observed among the 32,731 subjects enrolled for participation in the clinical validation cohort. These results support the safety of SimpleScreen CRC.

b. Effectiveness Results

Effectiveness evaluations for SimpleScreen CRC were pre-defined as follows:

Table 14: PREEMPT CRC Objectives

Endpoint	Definition
CRC Sensitivity	Proportion of participants with a positive test result among those who had a diagnosis of colorectal cancer
ACN Specificity	Proportion of participants with a negative test result among those who had non-advanced precancerous lesions or negative findings (non-neoplastic or no findings)
APL Sensitivity	Proportion of participants with a positive test result among those who had a diagnosis of advanced precancerous lesions

Primary Performance Objectives

The primary effectiveness evaluation was performed for the 27,010 evaluable subjects. The primary clinical performance measures included sensitivity of the SimpleScreen CRC test for CRC, and specificity among participants not having ACN (CRC or APL) using colonoscopy with histopathology as the clinical ground truth. The distribution of SimpleScreen Blood Test results by histopathological category is summarized in **Table 15**.

Table 15: Distribution of SimpleScreen Blood Test Result by Histopathological Category, n=27,010

SimpleScreen Blood Test Result	Colonoscopy/Histopathology Lesion Category Grouping			Total
	CRC (Category 1)	APL (Category 2)	NAPL and NEG (Category 3-5 and 6)	
Positive	57	321	2065	2443
Negative	15	2246	22306	24567
Total	72	2567	24371	27010

Sensitivity for CRC was 79.2% (57/72), with a 2-sided confidence interval of 68.4%-86.9% (**Table 16**); Specificity for ACN was 91.5%, with a 2-sided confidence interval of 91.2%-91.9% (**Table 16**).

Table 16: Measures of Clinical Performance: Sensitivity and Specificity

Primary Endpoint	Estimate (%), (n/N), 95% CI
CRC Sensitivity	79.2%, (57/72) 68.4%-86.9%
ACN Specificity	91.5%, (22306/24371) 91.2%-91.9%
CRC Specificity	91.1%, (24552/26938) 90.8% - 91.5%

In addition, the primary effectiveness results were calculated adjusting the results to reflect the 2020 U.S. census age and sex distribution (Census-adjusted). Compared to the U.S. census age and sex distribution, the evaluable cohort included a higher proportion of participants aged 50 to 54 years, a lower proportion of those aged 75 years and greater, and a higher proportion of females (**Table 17**). A pre-specified analysis was conducted to adjust clinical performance to the age and sex distribution of the U.S. census population. U.S. census-adjusted sensitivity for CRC was 81.1%, specificity for ACN was 90.4%, NPV for ACN was 90.5%, and PPV for ACN was 15.5%. The comparison between observed and U.S. census-adjusted results is summarized in **Table 18**.

Table 17: Age and Sex Distribution

Sex	Age Group	U.S. Estimate[1]	Evaluable Cohort (27,010)
Male	45 to 49	7.67%	4.74%
	50 to 54	7.78%	14.12%
	55 to 64	15.83%	14.53%
	65 to 74	11.44%	9.39%
	75 and older	5.50%	1.41%
Female	45 to 49	7.66%	6.25%
	50 to 54	7.81%	18.83%
	55 to 64	16.50%	17.77%
	65 to 74	12.81%	11.36%
	75 and older	7.01%	1.60%

1: U.S. census age and sex data were referenced from published data tables[3]

Table 18: Comparison of Observed and Census Adjusted performance

Primary Endpoint	Observed %, 2-sided 95% CI, (n/N)	U.S. Census adjusted[1], %, 2-sided 95% CI[1], (n/N)
CRC Sensitivity	79.2%, 68.4%-86.9% (57/72)	81.1%, 71.3%-88.1% (66.43/81.91)
ACN Specificity	91.5%, 91.2%-91.9% (22306/24371)	90.4%, 90.0%-90.7% (21938.03/24280.65)

1: U.S. Census age and sex data were referenced from published data tables[3]

Secondary Performance Objective

The secondary effectiveness objective was APL sensitivity, which was 12.5% (95% CI 11.3%-13.8%).

When adjusted to the age and sex distribution of the U.S. census population, sensitivity for APL was 13.7% (95% CI 12.4%-15.0%).

Table 19: Secondary Effectiveness Objective

Secondary Objective	Observed %, 2-sided 95% CI, (n/N)	U.S. Census adjusted[1] %, 2-sided 95% CI, (n/N)
APL Sensitivity	12.5% 11.3%-13.8%, (321/2567)	13.7% 12.4%-15.0%, (362.03/2647.44)

1: U.S. Census age and sex data were referenced from published data tables[3]

Positive and Negative Predictive Values

The predictive values of the test indicate the chance of disease for the subjects with positive or negative test result, given the disease prevalence.

- The positive predictive value (PPV) for CRC is a fraction of patients with CRC among the patients with positive SimpleScreen CRC test results.
- The PPV for APL is a fraction of patients with APL among the patients with positive SimpleScreen CRC test results.
- A negative predictive value (NPV) for CRC is a fraction of patients without CRC among the patients with negative SimpleScreen CRC test results and 1-NPV is a fraction of patients with CRC among the patients with negative SimpleScreen CRC test results

- A negative predictive value for ACN is a fraction of patients without ACN (CRC or APL) among the patients with negative SimpleScreen CRC 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.

The observed CRC prevalence in clinical study was 0.27%, and APL prevalence was 9.5%. At these prevalence values, the PPVs indicate that individuals with a positive test result have a 2.3% chance of having CRC, and 13.1% chance of having APLs. The NPVs indicate that individuals with a negative test result have a 0.06% (1- CRC NPV) chance of having CRC, and a 9.1% (1- APL NPV) chance of having APLs. Results are summarized in **Table 20**. The CRC positive likelihood ratio is 8.94, and the negative likelihood ratio is 0.229.

Table 20: PPV and NPV of CRC, APL or ACN

Age	Prevalence of CRC	Prevalence of APL	Percent Positive SimpleScreen Results	CRC PPV	APL PPV	CRC NPV	ACN NPV
45-49	0.24% (7/2968)	8.32% (247/2968)	5.86%	4.02%	14.37%	100.00%	92.05%
50-59	0.26% (31/12096)	9.06% (1096/12096)	7.27%	2.62%	12.29%	99.93%	91.12%
60-69	0.23% (21/9149)	10.25% (938/9149)	10.45%	1.67%	13.08%	99.94%	90.02%
70-79	0.45% (12/2661)	10.33% (275/2661)	15.30%	2.46%	14.99%	99.91%	90.42%
80-84	0.74% (1/136)	8.09% (11/136)	19.85%	3.70%	7.41%	100.00%	91.74%
Observed	0.27% (72/27010)	9.50% (2567/27010)	9.04%	2.33%	13.14%	99.94%	90.80%
Age Adjusted	0.30%	9.48%	10.03%	2.51%	13.02%	99.94%	90.85%

Subgroup Analyses

The SimpleScreen CRC performance measures in the primary analysis data set were also evaluated in pre-specified subgroups, including baseline demographic characteristics, cancer stage, lesion characteristics, and histopathological subgroups.

Table 21: Sensitivity and Specificity of SimpleScreen CRC: Cancer Stage and Histopathological Subgroups

Colonoscopy/histopathology	Sensitivity %, 95% CI (n/N)
Colorectal cancer	79.2%, 68.4%-86.9% (57/72)
Stage I	57.1%, 39.1%-73.5% (16/28)
Stage II	100.0%, 79.6%-100.0% (15/15)
Stage III	82.4%, 59.0%-93.8% (14/17)
Stage IV	100.0%, 74.1%-100.0% (11/11)
Stage unknown	100.0%, 20.7%-100.0% (1/1)
Advanced precancerous lesions	12.5%, 11.3%-13.8% (321/2567)
Adenoma with carcinoma in situ or high-grade dysplasia, any size (collectively HGD)	29.1%, 21.4%-38.2% (32/110)
Adenoma, villous growth pattern (≥25%), any size	15.2%, 12.5%-18.4% (86/564)
Adenoma ≥1.0 cm in size	11.5%, 10.0%-13.2% (169/1470)
Sessile serrated lesion with or without cytological dysplasia ≥1.0 cm	7.6%, 5.4%-10.7% (30/394)
Traditional serrated adenoma, any size	13.8%, 5.5%-30.6% (4/29)
Colonoscopy/histopathology	**Specificity %, 95% CI (n/N)**
CRC specificity categories (category 2-6)	91.1%, 90.8%-91.5% (24552/26938)
ACN specificity categories (category 3-6)	91.5%, 91.2%-91.9%

	(22306/24371)
Non-advanced precancerous lesions (category 3-5)	91.6%, 91.0%-92.1% (8184/8936)
≥5 adenomas, all <1.0 cm in size, non-advanced (category 3)	90.8%, 86.0%-94.1% (178/196)
3 to 4 adenomas, all <1.0 cm in size, non-advanced (category 4)	91.5%, 89.3%-93.2% (696/761)
1 to 2 adenomas, all <1.0 cm in size, non-advanced; or HP ≥1.0 cm; or all SSL <1.0 cm, and HP <1.0 cm not in sigmoid or rectum (category 5)	91.6%, 91.0%-92.2% (7310/7979)
Negative findings (category 6)*	91.5%, 91.0%-91.9% (14122/15435)
No findings (category 6.2)*	91.5%, 91.0%-91.9% (11383/12446)

*Refer to **Table 11**

Table 22: Sensitivity and Specificity of SimpleScreen CRC: Lesion Characteristics

Characteristic	CRC		APL		ACN Specificity	
	Sensitivity %, (n/N)	%Total (N/72)	Sensitivity %, (n/N)	%Total (N/2,567)	Specificity %, (n/N)	%Total (N/24,371)
Index lesion size (mm)						
≤5	20.0% (1/5)	6.9%	13.4% (9/67)	2.6%	91.5% (7584/8286)	34.0%
6-9	33.3% (1/3)	4.2%	12.1% (17/140)	5.5%	91.7% (3118/3399)	13.9%
10-19	50.0% (5/10)	13.9%	11.3% (230/2031)	79.1%	92.3% (203/220)	0.9%
20-29	90.9% (10/11)	15.3%	16.5% (37/224)	8.7%	88.2% (15/17)	0.1%
≥30	92.7% (38/41)	56.9%	25.0% (25/100)	3.9%	100.0% (3/3)	0.0%
No index lesion or no index lesion size recorded	100.0% (2/2)	2.8%	60.0% (3/5)	0.2%	91.5% (11,383/12,446)	51.1%

Characteristic	CRC		APL		ACN Specificity	
Index lesion location						
Proximal	100.0% (11/11)	15.3%	13.1% (190/1451)	56.5%	91.4% (5664/6196)	25.4%
Distal	66.7% (22/33)	45.8%	10.6% (85/804)	31.3%	92.1% (3634/3946)	16.2%
Rectum	85.7% (24/28)	38.9%	14.8% (46/311)	12.1%	91.1% (1619/1777)	7.3%
No index lesion or no index lesion location recorded	n/a (0/0)	0.0%	0.0% (0/1)	0.0%	91.5% (11,389/12,452)	51.1%

Table 23: Sensitivity and Specificity of SimpleScreen CRC: Demographic Characteristics

Characteristic	CRC Sensitivity %, (n/N)	APL Sensitivity %, (n/N)	ACN Specificity %, (n/N)
Sex			
Female	80.0% (28/35)	12.1% (140/1154)	92.1% (12,789/13,887)
Male	78.4%, (29/37)	12.8% (181/1413)	90.8% (9517/10,484)
Age (years)			
45-49	100.0% (7/7)	10.1% (25/247)	94.8% (2572/2714)
50-54	66.7% (12/18)	8.6% (66/769)	93.5% (7588/8112)
55-64	83.3% (20/24)	12.7% (112/885)	91.5% (7149/7816)
65-74	78.9% (15/19)	17.7% (104/587)	87.8% (4389/4998)
≥75	75.0% (3/4)	17.7% (14/79)	83.2% (608/731)
Race			
American Indian or Alaska Native	n/a (0/0)	11.1% (1/9)	87.0% (60/69)
Asian	78.6% (11/14)	8.3% (11/132)	88.4% (1975/2235)
Black or African American	85.7% (6/7)	13.1% (33/251)	92.8% (2581/2780)
Native Hawaiian or Other Pacific Islander	n/a (0/0)	9.1% (1/11)	93.4% (57/61)
White	77.1% (37/48)	12.9% (260/2023)	91.7% (16,175/17,636)
More than one race	n/a (0/0)	0.0% (0/20)	87.9% (102/116)
Other/Unknown	100.0% (3/3)	12.4% (15/121)	92.0% (1356/1474)
Ethnicity			
Hispanic or Latino	77.8% (7/9)	10.4% (23/221)	90.3% (2672/2959)
Non-Hispanic or Latino	80.6% (50/62)	12.4% (274/2208)	91.6% (18,467/20,151)

Characteristic	CRC Sensitivity %, (n/N)	APL Sensitivity %, (n/N)	ACN Specificity %, (n/N)
Unknown	0.0% (0/1)	17.4% (24/138)	92.5% (1167/1261)

MEANING OF SYMBOLS

REF	Catalog Number
	Use By
SN	Serial Number
LOT	Batch Code
IVD	In Vitro Diagnostic Medical Device
	Content Sufficient for Number of Tests Specified
	Do Not Re-use
	Biological Risk
	Consult Instructions for Use
	Temperature Limit
	Manufacturer
Rx Only	By Prescription Only
	Warning or Caution Information

TRADEMARKS

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CONTACT INFORMATION

For any questions about the test, please contact Freenome Client Services at 833-990-6185 or email support@freenome.com.

REFERENCES

1. Dahlhamer JM, Zammitti EP, Ward BW, Wheaton AG, Croft JB. Prevalence of inflammatory bowel disease among adults aged ≥18 years — united states, 2015. *MMWR Morb Mortal Wkly Rep*. 2016;65(42):1166-1169. doi:10.15585/mmwr.mm6542a3

2. Shaukat A, Burke CA, Chan AT, et al. Clinical Validation of a Circulating Tumor DNA–Based Blood Test to Screen for Colorectal Cancer. *JAMA*. 2025;334(1):56-63. doi:10.1001/jama.2025.7515

3. U.S. Census Bureau, Population Division. Annual Estimates of the Resident Population for Selected Age Groups by Sex for the United States: April 1, 2020 to July 1, 2023 (NC-EST2023-AGESEX). June 2024. Accessed July 29, 2025. https://www2.census.gov/programs-surveys/popest/tables/2020-2023/national/asrh/nc-est2023-agesex.xlsx

4. Nam J. Confidence limits for the ratio of two binomial proportions based on likelihood scores: non-iterative method. *Biom J*. 1995;37(3):375-379. doi:10.1002/bimj.4710370311

5. Liles EG, Perrin N, Rosales AG, et al. Performance of a quantitative fecal immunochemical test for detecting advanced colorectal neoplasia: a prospective cohort study. *BMC Cancer*. 2018;18(1):509. doi:10.1186/s12885-018-4402-x

6. Ahlquist DA, Sargent DJ, Loprinzi CL, et al. Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia. *Ann Intern Med*. 2008;149(7):441-450. doi:10.7326/0003-

4819-149-7-200810070-00004

7. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. *N Engl J Med*. 2014;370(14):1287-1297. doi:10.1056/NEJMoa1311194

8. Redwood DG, Asay ED, Blake ID, et al. Stool DNA Testing for Screening Detection of Colorectal Neoplasia in Alaska Native People. *Mayo Clinic Proceedings*. 2016;91(1):61-70. doi:10.1016/j.mayocp.2015.10.008

9. Shapiro JA, Bobo JK, Church TR, et al. A Comparison of Fecal Immunochemical and High-Sensitivity Guaiac Tests for Colorectal Cancer Screening. *Am J Gastroenterol*. 2017;112(11):1728-1735. doi:10.1038/ajg.2017.285

10. Cooper JA, Parsons N, Stinton C, et al. Risk-adjusted colorectal cancer screening using the FIT and routine screening data: development of a risk prediction model. *Br J Cancer*. 2018;118(2):285-293. doi:10.1038/bjc.2017.375

11. U.S. Food & Drug Administration. Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Human Papillomaviruses: Guidance for Industry and Food and Drug Administration Staff. September 15, 2017. Accessed July 29, 2025. https://www.fda.gov/media/92930/download