SimpleScreen™ CRC

IVD Rx ONLY

Freenome, LLC | 3300 Marina Blvd | Brisbane, California 94005
freenome.com | support@freenome.com | 833-990-6185

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1. 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.

2. 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.

3. 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 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, and Turcot's/Crail's syndrome, Cronkhite-Canada syndrome, or Neurofibromatosis.

4. 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 CRC 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 CRC after age 60.
- Non-inferiority or superiority of SimpleScreen CRC sensitivity as compared to other recommended screening methods for CRC or APLs 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 (part number K00014) for precautions and limitations specific to the collection and shipping of blood samples.

5. SimpleScreen CRC Overview

SimpleScreen™ CRC is a qualitative in vitro diagnostic (IVD) blood test intended to detect molecular signals (methylation) associated with colorectal cancer (CRC) from whole blood samples collected from individuals at average risk of the disease. Samples are collected via a standard blood draw from patients at average risk for CRC and processed at Freenome's CLIA-certified laboratory. The resulting data are analyzed by proprietary software that includes an artificial intelligence/machine learning (AI/ML)-enabled classification algorithm. Results are reported as "Positive" or "Negative."

The SimpleScreen CRC blood test can be completed during any patient visit, alongside other blood tests. The test can be considered in a manner similar to guideline-recommended non-invasive CRC screening modalities and is not a replacement for diagnostic colonoscopy or for surveillance colonoscopy in high-risk individuals.

A positive result may indicate the presence of colorectal cancer or advanced precancerous lesions and should be followed by colonoscopy. A negative result indicates the test did not detect a signal associated with colorectal cancer.. A negative test result does not guarantee the absence of colorectal cancer or advanced precancerous lesions. Healthcare providers should work with individuals to continue guideline-recommended CRC screening programs.

In the PREEMPT CRC® study, SimpleScreen CRC demonstrated a sensitivity of 79.2% for CRC and a specificity of 91.5% for advanced colorectal neoplasia (ACN). SimpleScreen CRC has a false positive rate of 10%, meaning that one out of 10 people who do not have ACN (colorectal cancer or advanced

precancerous lesions) will have a false positive test result. SimpleScreen CRC has a false negative rate of 21% for colorectal cancer, meaning that 21 out of 100 people who have colorectal cancer will incorrectly receive a negative test result (Table 4).

In the PREEMPT CRC clinical study, SimpleScreen CRC's sensitivity by stage was 57.1% for Stage I CRC, 100% for Stage II CRC, 82.4% for Stage III CRC, and 100% for Stage IV CRC.

Performance was also evaluated after adjusting for the U.S. Census age and sex distribution. When adjusting performance to match the age and sex distribution of the 2020 U.S. Census population, CRC sensitivity was 81.1%, and specificity was 90.4% for ACN. Sensitivity by stage was 63.5% for Stage I CRC, 100% for Stage II CRC, 80.5% for Stage III CRC, and 100% for Stage IV CRC. Sensitivity for advanced precancerous lesions was 13.7%. See Table 6 summarizing observed and Census adjusted performance.

When discussing CRC screening with patients, providers should share the benefits and risks of all screening modalities and work to find the option that is most appropriate for the patient's overall health, prior screening history, personal preference, and schedule constraints.

Colonoscopy should be discussed with all patients, given its ability to detect and potentially remove colorectal lesions.

6. Colorectal Cancer Overview

CRC is the fourth-most commonly diagnosed cancer, occurring in approximately 150,000 individuals annually, and is the second-most common cause of cancer death, claiming 50,000 lives per year.[1] When CRC is found as an early, localized disease, the 5-year survival rate is 91%, but this rate falls to 13% for metastatic disease.[1] Despite this, nearly 37% of eligible Americans are not up to date on their CRC screening.[2]

Several studies report that individuals who decline conventional screening methods prefer blood-based testing,[3,4] and an increase in screening rates when blood-based testing is offered to patients who previously declined screening.[3,5,6] The actual number of CRC cases identified through screening depends on both the ability of the test to detect the disease (sensitivity) and the willingness of the patient to complete a particular screening test (adherence).

7. Device Description

SimpleScreen CRC is an in vitro diagnostic blood test that qualitatively detects molecular signals associated with CRC. SimpleScreen CRC identifies DNA methylation patterns associated with CRC from circulating cell-free DNA. Samples are collected via a standard blood draw from patients at average risk for CRC and processed at Freenome's CLIA-certified laboratory. The resulting data are analyzed by proprietary software that includes an AI/ML-enabled classification algorithm. Results are reported as

"Positive" or "Negative." A patient with a positive result should be followed by colonoscopy. A patient with a negative result should continue participating in colorectal cancer screening programs. Note that 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.

8. Clinical Study Overview

The clinical performance of SimpleScreen CRC test was evaluated in a prospective, blinded, multicenter study (Prevention of Colorectal Cancer Through Multiomics Blood Testing, PREEMPT CRC).[7] The primary objective of the study was to evaluate the sensitivity of SimpleScreen CRC in detecting colorectal cancer, and specificity for ACN (colorectal cancer or advanced precancerous lesions) in individuals at average risk for CRC.

Study design

The PREEMPT CRC study[7] participants were screen-eligible individuals who were aged 45 to 85 years within 30 days of enrollment, asymptomatic, at average risk for CRC, and willing to undergo blood collection, and a standard-of-care screening colonoscopy. The standard-of-care colonoscopy was required to be performed within 120 days after the blood draw.

Blood samples were sent to the Freenome lab for processing, testing, and storage. The clinical outcome of participants was determined by colonoscopy with histopathology. The lesion of greatest clinical significance was used to categorize each participant (Table 1). Central pathology reviews were conducted for all CRCs and certain subtypes of APLs and negative findings. Lab testing was performed independent of the clinical findings, and all participants, site staff, and central pathologists were blinded to the results.

The clinical validation population consisted of 27,010 participants who had valid blood test results and confirmed clinical findings. There were no serious adverse events reported in the study.

Table 1. Histopathology Classification

Category	Findings
1	**CRC**
	1.0. CRC, all Stages (I-IV)
2	**APL**
	2.1. Adenoma with carcinoma in situ or high-grade dysplasia, any size
	2.2. Adenoma, villous growth pattern (≥25%), any size
	2.3. Adenoma ≥1.0 cm in size
	2.4. Serrated lesion, ≥ 1.0 cm (includes sessile serrated adenoma/polyp)
	2.5. Traditional serrated adenoma (TSA), any size
3	**Non-advanced precancerous lesions**

Category	Findings
	3.0. ≥5 adenomas, all <1.0 cm in size, non-advanced
4	**Non-advanced precancerous lesions**
	4.0. 3 to 4 adenomas, all <1.0 cm in size, non-advanced
5	**Non-advanced precancerous lesions**
	5.1. 1 to 2 adenomas, all <1.0 cm in size, non-advanced
	5.2. Hyperplastic polyps ≥1.0 cm
	5.3. All SSL <1.0 cm, and HP <1.0 cm not in sigmoid or rectum
6	**Negative findings**
	6.1. Negative and other findings upon histopathological review
	6.2. No findings on colonoscopy, no histopathological review

NAPL= non-advanced precancerous lesion; NEG = negative

Clinical Performance Measures

Primary performance measures included sensitivity for CRC and specificity for ACN. A secondary measure was sensitivity for APLs. Additional performance outcomes included positive predictive value (PPV) for CRC and APL and negative predictive value (NPV) for CRC and ACN, using colonoscopy with histopathology as the clinical ground truth.

The pre-defined objectives for SimpleScreen CRC effectiveness evaluation are listed in Table 2.

Table 2: Clinical Study Endpoints

Endpoint	Definition
CRC Sensitivity	Proportion of participants with a positive test result among those who had a diagnosis of CRC
ACN Specificity	Proportion of participants with a negative test result among those who had NAPL or NEG findings (non-neoplastic or no findings)
APL Sensitivity	Proportion of participants with a positive test result among those who had a diagnosis of APLs

Sensitivity for CRC (assessed on subjects in category 1 in Table 1) was considered acceptable if the lower bound of the 2-sided 95% confidence interval (CI) exceeds 65.0%.

Specificity for ACN (assessed on subjects in categories 3-6 in Table 1) was considered acceptable if the lower bound of the 2-sided 95% CI exceeds 85.0%.

Demographics and Baseline Characteristics

The demographics and baseline characteristics of the evaluable dataset are summarized in Table 3.

Table 3. Demographics Characteristics

	Evaluable dataset (N=27,010)
Age (years)	
Mean (SD)	58.1 (8.2)
Median (range)[a]	57.0 (45.0, 86.0)
Age group (years), n (%)	
45–49	2968 (11.0)
50–54	8899 (32.9)
55–64	8725 (32.3)
65–74	5604 (20.7)
75+	814 (3.0)
Sex, n (%)	
Female	15,076 (55.8)
Male	11,934 (44.2)
Race, n (%)	
American Indian or Alaska Native	78 (0.3)
Asian	2381 (8.8)
Black or African American	3038 (11.2)
Native Hawaiian or Other Pacific Islander	72 (0.3)
White	19,707 (73.0)
More than one reported	136 (0.5)
Other/Unknown	1598 (5.9)
Ethnicity	
Hispanic or Latino	3189 (11.8)
Not Hispanic or Latino	22,421 (83.0)
Unknown	1400 (5.2)
BMI (kg/m^2)	
Mean (SD)	29.4 (6.4)
Median	28.3

[a]Age was recorded based on year of birth; fractional age was not calculated. Per eligibility, the study population included participants aged 45 to 85, within one month of enrollment. Participants outside this age range were confirmed by the respective sites to meet the age eligibility at time of enrollment.

Study Results

The co-primary and secondary performance measures for the clinical study are summarized in Table 4 below. The sensitivity for CRC was 79.2% (95% CI, 68.4% to 86.9%). For subjects without a diagnosis of CRC or APLs, the specificity was 91.5% (95% CI, 91.2% to 91.9%). The sensitivity for APLs was 12.5% (95% CI, 11.3% to 13.8%).

Table 4: Performance Measures

Performance Measures	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%
APL Sensitivity	12.5%, (321/2567) 11.3%-13.8%

The primary and secondary performance measures were additionally evaluated after 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 5). To evaluate the population-level accuracy and representativeness of observed test performance, 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%, and specificity for ACN was 90.4%. The comparison between observed and Census-adjusted results is summarized in Table 6 below.

Table 5: Age and Sex Distribution

Sex	Age Group	U.S. Estimate1	Evaluable Dataset (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%

1U.S. Census age and sex data were referenced from published data tables

Table 6. Comparison of Observed and Census-Adjusted Performance of SimpleScreen CRC

Performance Metric	Evaluable Dataset (N=27,010), 2-sided 95% CI, (n/N)	
	Observed	Census-adjusted[a]
Sensitivity for CRC	79.2%, 68.4%-86.9% (57/72)	81.1%, 71.3%-88.1% (66.43/81.91)
Specificity for ACN	91.5%, 91.2%-91.9%	90.4%, 90.0%-90.7%

Performance Metric	Evaluable Dataset (N=27,010), 2-sided 95% CI, (n/N)	
	Observed	Census-adjusted[a]
	(22,306/24,371)	(21,938.03/24,280.65)
Sensitivity for APLs	12.5%, 11.3%-13.8% (321/2567)	13.7%, 12.4%-15.0% (362.03/2647.44)

[a]U.S. Census age and sex data were obtained from U.S. Census Bureau.[9]

Subgroup Analyses

The sensitivity and specificity of SimpleScreen CRC in the clinical study are reported by age, sex, race and ethnicity in Table 7. There was no substantial difference in sensitivity for CRC by demographic characteristics. The specificity for ACN correlated inversely with age. The sensitivity for APLs correlated with age (Table 7).

Table 7. Sensitivity and Specificity by Demographic Characteristics

	Sensitivity for CRC (N=72) (n/N)	Specificity for ACN (N=24,371) (n/N)	Sensitivity for APLs (N=2567) (n/N)
Sex			
Female	80.0% (28/35)	92.1% (12,789/13,887)	12.1% (140/1154)
Male	78.4% (29/37)	90.8% (9517/10,484)	12.8% (181/1413)
Age (years)			
45-49	100.0% (7/7)	94.8% (2572/2714)	10.1% (25/247)
50-54	66.7% (12/18)	93.5% (7588/8112)	8.6% (66/769)
55-64	83.3% (20/24)	91.5% (7149/7816)	12.7% (112/885)
65-74	78.9% (15/19)	87.8% (4389/4998)	17.7% (104/587)
≥75	75.0% (3/4)	83.2% (608/731)	17.7% (14/79)
Race			
American Indian or Alaska Native	- (0/0)	87.0% (60/69)	11.1% (1/9)
Asian	78.6% (11/14)	88.4% (1975/2235)	8.3% (11/132)
Black Or African American	85.7% (6/7)	92.8% (2581/2780)	13.1% (33/251)
Native Hawaiian or Other Pacific Islander	- (0/0)	93.4% (57/61)	9.1% (1/11)
White	77.1% (37/48)	91.7% (16,175/17,636)	12.9% (260/2023)
More than one race	- (0/0)	87.9% (102/116)	0.0% (0/20)
Other/Unknown	100.0% (3/3)	92.0% (1356/1474)	12.4% (15/121)
Ethnicity			
Hispanic or Latino	77.8% (7/9)	90.3% (2672/2959)	10.4% (23/221)
Non-Hispanic or Latino	80.6% (50/62)	91.6% (18,467/20,151)	12.4% (274/2208)

	Sensitivity for CRC (N=72) (n/N)	Specificity for ACN (N=24,371) (n/N)	Sensitivity for APLs (N=2567) (n/N)
Unknown	0.0% (0/1)	92.5% (1167/1261)	17.4% (24/138)

The sensitivity and specificity of SimpleScreen CRC from the clinical study are reported by Stage and Histopathological Subgroup in Table 8. Sensitivity of SimpleScreen CRC by lesion characteristics is reported in Tables 8 and 9. There was one case of unstaged cancer, which was detected by the test. Sensitivity for CRC increased with larger lesion size.

Table 8: Sensitivity and Specificity of SimpleScreen CRC: Cancer Stage and Histopathological Subgroups

Colonoscopy/histopathology	Sensitivity %, 2-sided 95% CI, (n/N)
CRC	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)
APLs	12.5%, 11.3%-13.8% (321/2567)
Adenoma with CIS or HGD, 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 %, 2-sided 95% CI, (n/N)**
No CRC (category 2 - 6)	91.1%, 90.8%-91.5% (24,552/26,938)
No ACN (category 3 - 6)	91.5%, 91.2%-91.9% (22,306/24,371)
Non-advanced precancerous lesions (category 3 - 5)	91.5%, 90.9%-92.2% (6655/7270)
≥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% (14,122/15,435)
No findings (category 6.2)	91.5%, 91.0%-91.9% (11,383/12,446)

[a]Stage was defined using the American Joint Committee on Cancer Staging System, 8th Edition.

The sensitivity for APLs with HGD was 29.1% (95% CI, 21.4% to 38.2%). APLs with HGD are lesions associated with 4.3 and 13.2 greater odds of developing advanced adenomas and CRC, respectively.[8]

Table 9. Sensitivity and Specificity by Lesion Characteristics

	Sensitivity for CRC (N=72) %, 2-sided 95% CI, (n/N)	**Specificity for ACN (N=24,371) %, 2-sided 95% CI, (n/N)**
Index lesion size (mm)[a]		
≤5	20.0%, 3.6%-62.4% (1/5)	91.5%, 90.9%-92.1% (7584/8286)
6-9	33.3%, 6.1%-79.2% (1/3)	91.7%, 90.8%-92.6% (3118/3399)
10-19	50.0%, 23.7%-76.3% (5/10)	92.3%, 88.0%-95.1% (203/220)
20-29	90.9%, 62.3%-98.4% (10/11)	88.2%, 65.7%-96.7% (15/17)
≥30	92.7%, 80.6%-97.5% (38/41)	100.0%, 43.9%-100.0% (3/3)
No index lesion or no index lesion size recorded	100.0%, 34.2%-100% (2/2)	91.5%, 91.0%-91.9% (11,383/12,446)
Index lesion location[b]		
Proximal	100%, 74.1%-100% (11/11)	91.4%, 90.7%-92.1% (5664/6196)
Distal	66.7%, 49.6%-80.2% (22/33)	92.1%, 91.2%-92.9% (3634/3946)
Rectum	85.7%, 68.5%-94.3% (24/28)	91.1%, 89.7%-92.3% (1619/1777)
No index lesion or no index lesion location recorded	- (0/0)	91.5%, 91.0%-91.9% (11,389/12,452)

[a]The CRC sizes were reported based on histopathology or surgical reports. The size information was not available for two CRCs, which were detected by the blood-based test.
[b]The proximal colon was considered to include the ileocecal valve, cecum, ascending colon, hepatic flexure, and transverse colon. The distal colon was considered to include the splenic flexure, descending colon, and sigmoid colon. The rectosigmoid was considered as part of the rectum.

There were no pre-specified performance targets for the exploratory analyses, including subgroup analyses, and the study was neither powered nor alpha-controlled for these outcomes.

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 10. The CRC positive likelihood ratio is 8.94, and the negative likelihood ratio is 0.229.

Table 10: 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	Prevalence of CRC	Prevalence of APL	Percent Positive SimpleScreen Results	CRC PPV	APL PPV	CRC NPV	ACN NPV
Age Adjusted	0.30%	9.48%	10.03%	2.51%	13.02%	99.94%	90.85%

9. Patient Testing with SimpleScreen CRC

To order SimpleScreen CRC, a test requisition form must be completed and signed by the ordering physician or other authorized medical professional. Ordering is available through a paper requisition form returned via e-fax or in the blood collection kit, the provider online portal found at provider.freenome.com, or an electronic health records integration at some sites.

Blood samples for SimpleScreen CRC must be collected and shipped using the Freenome Blood Collection Kit (BCK). 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 further details about collecting and shipping blood samples to the Freenome Clinical Laboratory, refer to the Freenome BCK Instructions For Use. To order additional Freenome BCKs, contact Freenome Client Services.

Results are delivered back to the physician in about two weeks. For any questions about SimpleScreen CRC or the BCK, contact Freenome Client Services at 833-990-6185 or support@freenome.com.

10. Interpretation of Results

A test report will be returned to the ordering healthcare provider with a result of positive or negative. Patients are advised to talk with their healthcare provider about their SimpleScreen CRC results. In some cases, the test may not produce a result, which will be reported as “No Result.” If this occurs, the ordering physician will be contacted, and the patient may be asked to provide another blood sample.

A positive result indicates a signal was detected that may suggest the presence of colorectal cancer or advanced precancerous lesions. A colonoscopy evaluation is necessary to determine whether CRC is present.

A negative result indicates the test did not detect a signal associated with colorectal cancer or advanced precancerous lesions. A negative test result does not guarantee absence of colorectal cancer or advanced precancerous lesions.

The sensitivity of SimpleScreen CRC for detection of colorectal cancer is 79.2%. This means that approximately 21% of patients with CRC will have a false negative SimpleScreen CRC test result, meaning that colorectal cancer was actually present but was not detected by the test.

The specificity of the SimpleScreen CRC in detecting Advanced Colorectal Neoplasia (which includes both colorectal cancer and advanced precancerous lesions) is 91.5%. This means that approximately 8 of individuals without any colorectal neoplasia will have positive results that were a false positive, meaning that neither colorectal cancer nor advanced precancerous lesions were actually present.

The sensitivity of the SimpleScreen CRC for detecting advanced precancerous lesions is 12.5%, and 87.5% of patients with advanced precancerous lesions will have a false negative result. The SimpleScreen CRC is not intended to be used to screen for the presence of advanced precancerous lesions.

Patients should be referred for a colonoscopy evaluation to confirm whether colorectal cancer or an advanced adenoma is present following a positive result. Patients with a negative result should continue participating in colorectal cancer screening programs, at the appropriate guideline recommended intervals.

11. Patient Education Through Shared Decision-Making

It is important to engage in shared decision-making when discussing CRC screening options with patients. Research shows that presenting multiple choices for CRC screening increases overall screening rates; therefore shared decision-making in which multiple CRC screening options are introduced can help drive more screening and better outcomes.

When talking with patients about screening for CRC, providers should share information about all screening options, including their benefits and risks. Patients should choose the screening test that is most appropriate based on their overall health, prior screening history, personal preference, and schedule constraints.

SimpleScreen CRC can be considered in a manner similar to guideline-recommended non-invasive CRC screening options.

As part of the shared decision-making process, it's important to communicate the following information to all patients:

- Colonoscopy should be discussed with all patients, given the ability to detect and potentially remove colorectal lesions during the procedure.
- Detection of early-stage disease is important in the reduction of colorectal cancer mortality. SimpleScreen CRC has limited ability to prevent the development of CRC given the current data available. SimpleScreen CRC has limited ability to detect APLs, the precursor lesions that over time, may develop into CRC. In the clinical study, 87.5% of individuals with APL incorrectly received a negative result. This may impact the ability to prevent the development of CRC.
- A negative SimpleScreen CRC test does not preclude a CRC or APL diagnosis and patients should continue participating in CRC screening programs. A positive SimpleScreen CRC test should be followed by a colonoscopy for the patient
- SimpleScreen CRC test did a better job identifying Stage II, III, and IV colorectal cancer (100% of individuals with Stage II and Stage IV cancers were correctly identified, and 82.4% of individuals

with Stage III cancers were identified) than Stage I CRC (57.1% of individuals with these cancers were correctly identified with SimpleScreen CRC). This means that 42.9% of individuals with Stage I CRC were not identified with a SimpleScreen CRC test.

- 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%. 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%. These are lesions that may have been detected by a screening colonoscopy.

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

12. References

1. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. *CA Cancer J Clin*. 2025;75(1):10-45. doi:10.3322/caac.21871
2. Bandi P, Star J, Mazzitelli N, et al. Prevalence and Review of Major Modifiable Cancer Risk Factors, HPV Vaccination, and Cancer Screenings in the United States: 2025 Update. *Cancer Epidemiol Biomarkers Prev*. 2025;34(6):836-849. doi:10.1158/1055-9965.EPI-24-1835
3. Adler A, Geiger S, Keil A, et al. Improving compliance to colorectal cancer screening using blood and stool based tests in patients refusing screening colonoscopy in Germany. *BMC Gastroenterol*. 2014;14(1):183. doi:10.1186/1471-230X-14-183
4. Ioannou S, Sutherland K, Sussman DA, Deshpande AR. Increasing uptake of colon cancer screening in a medically underserved population with the addition of blood-based testing. *BMC Cancer*. 2021;21(1):966. doi:10.1186/s12885-021-08678-8
5. Liang PS, Zaman A, Kaminsky A, et al. Blood test increases colorectal cancer screening in persons who declined colonoscopy and fecal immunochemical test: a randomized controlled trial. *Clin Gastroenterol Hepatol*. 2023;21(11):2951-2957.e2. doi:10.1016/j.cgh.2023.03.036
6. Coronado GD, Jenkins CL, Shuster E, et al. Blood-based colorectal cancer screening in an integrated health system: a randomised trial of patient adherence. *Gut*. 2024;73(4):622-628. doi:10.1136/gutjnl-2023-330980
7. 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
8. Fairley KJ, Li J, Komar M, Steigerwalt N, Erlich P. Predicting the risk of recurrent adenoma and incident colorectal cancer based on findings of the baseline colonoscopy. *Clin Transl Gastroenterol*. 2014;5(12):e64. doi:10.1038/ctg.2014.11
9. 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)*.; 2024. https://www2.census.gov/programs-surveys/popest/tables/2020-2023/national/asrh/nc-est2023-agesex.xlsx
10. 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

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