DIACARTA

ColoScape™ Colorectal Cancer Mutation Detection Test

Improved Sensitivity for Early Colorectal Cancer Screening using FFPE and Plasma Samples

DEATH FROM COLORECTAL CANCER IS PREVENTABLE

Colorectal cancer is cancer that starts in the colon or the rectum. These cancers can also be named colon cancer or rectal cancer, depending on where they start. Colon cancer and rectal cancer are often grouped together because they have many features in common. Colorectal cancer is the third most common cancer in the world and in the U.S.

The trend for the death rate of colorectal cancer has dropped steadily over the years. The development of colorectal cancer takes about 15 years and the death from colorectal cancer is preventable with early diagnosis. Colorectal cancer screening is strongly suggested to get diagnosed early to prevent death.

According to the American Cancer Society, the guideline for colorectal screening is to start the colorectal cancer screening at age 50 (most recent changes to age 45).

Source: American Cancer Society

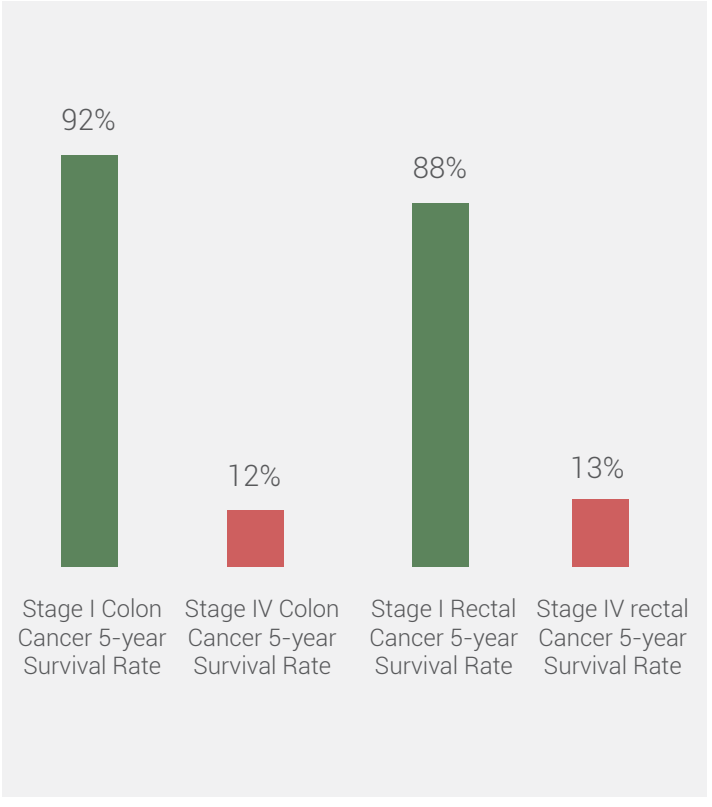
However, among the 98 million Americans aged 50 to 84, one-third of them are not screened for colorectal cancer for different reasons.

50,630
PEOPLE DIED
of colorectal cancer in 2018

97,220
NEW CASES
a year for colon cancer

43,030
NEW CASES
a year for rectal cancer

140,250
NEW CASES
a year for colorectal cancer



Source: American Cancer Society

CURRENT COLORECTAL CANCER DETECTION METHODS

As a gold standard for colon cancer screening, invasive colonoscopy not only identifies the polyps on the surface of the colon but removes them as well. However, part of the reasons that one-third of the population aged 50 to 84 is not using this screening tool is due to either the unpleasant experience for the preparation of colonoscopy or worries of medical complications caused by bleeding and infection during the process of colonoscopy.

The traditional non-invasive assays such as FIT assays are based on blood test using immunochemistry and their sensitivity is much lower (and therefore inaccurate) compared to the invasive tests.

Molecular diagnostics as a powerful non-invasive test has only recently been approved for colorectal screening. However, the current stool samples based tests are making it less inconvenient. Blood test is minimal invasive and well accepted. In addition, circulating cell-free DNA (cfDNA) in blood allows identification of cancer by detection of driver gene mutations with high sensitivities using techniques such as XNA clamps.

TESTS		DESCRIPTION	SENSITIVITY		SPECIFICITY
			COLORECTAL CANCER	ADVANCED ADENOMAS	
INVASIVE TESTS	Colonoscopy	Colonoscopy is the gold standard for colorectal cancer screening. It allows a doctor to examine closely the inside of the entire colon and rectum for polyps which could be an early sign of cancer and grow over time to develop cancer	95%	95%	90%
	Sigmoidoscopy	Examination of sigmoid colon (most distant part of colon) by means of a flexible tube inserted through the anus	~50% (95% in distal only)	~50% (95% in distal only)	92%
	CT Colonography	Computed tomography (CT) colonography or virtual colonoscopy uses special x-ray equipment to examine the large intestine for cancer and growths called polyp	96%	94%	86% - 96%
NON-INVASIVE TESTS	FIT	FIT (Fecal Immunochemical Test) is a test that checks for occult (hidden) blood in the stool. It only checks FIT hemoglobin	70%	22%	95%
	GFOBT (Hemoccult SENSA)	gFOBT (guaiac fecal occult blood test) is a test that checks for occult (hidden) blood in the stool	70%	24%	93%
	GFOBT (Hemoccult II)	gFOBT (guaiac fecal occult blood test) is a test that checks for occult (hidden) blood in the stool	40%	12%	98%
	ColoGuard®	ColoGuard® is an FDA approved test (2014) for colorectal cancer by checking gene mutations and methylations from stool DNA. 11 biomarkers 10 for stool DNA plus hemoglobin).	92%	69%	87%
	Epi procolon®	Epi proColon is a plasma DNA test for detection of a biomarker, methylated Septin9, associated with colorectal cancer.	68.2%	-	80%
	ColoScape™	ColoScape™ is a novel multigene mutation biomarker qPCR-based assay for qualitative detection of colorectal cancer (CRC) associated somatic mutations in the genes that are frequently mutated in colorectal cancer subjects and are responsible for aberrant colonic epithelial cell proliferation.	≥88.9%	66.6%	≥96%

INTRODUCING COLOSCAPE™ CRC MUTATION DETECTION TEST

XNA TECHNOLOGY

CE-MARKED

**ColoScape™ assay is CE-marked outside of US. It has not been cleared by FDA and it is intended to be used for research purpose only in the US.*

ColoScape™ Colorectal Cancer Mutation Detection Test is a novel highly-sensitive *in vitro* diagnostic assay using the qPCR-based multigene panel for the qualitative detection of colorectal cancer-associated gene mutations in liquid biopsy and FFPE tissue samples. The kit utilizes our XNA technology which leverages a sequence-specific clamp made by xeno-nucleic acid (XNA) to suppress PCR amplification of wild-type DNA template and selectively amplify only mutant DNA template, reaching sensitivity at 0.1% to 0.5% Variant Allele Frequency (VAF) with 10 ng DNA input. The detection kit identifies the presence or absence of mutations in the targeted regions of four colon cancer-associated genes.

The assay can be performed on qPCR instrumentation that is already available in hospital pathology laboratories. Unlike other colorectal cancer tests on the market, ColoScape™ provides a comprehensive profile of the key colorectal cancer 'driver' and 'resistance' mutations (gene variation landscape) and offers oncologists valuable information to evaluate targeted therapy options. ColoScape™ could also be used to complement existing colorectal cancer detection tests.

ColoScape™ is Optimal for Liquid Biopsy

With liquid biopsy gaining more traction, circulating cell-free DNA (cfDNA) in blood samples can be used for testing gene mutations as powerful cancer diagnostics tools. ColoScape™ assay is developed for colorectal cancer screening in the US and as a triage in Europe after FIT-positive test and before the colonoscopy examination. Preliminary testing data shows ColoScape™ assay has ≥88.9% sensitivity and specificity of ≥96% for stage I to IV colorectal cancer and 66.6% sensitivity for pre-cancer advanced adenomas.

Patented Colorectal Cancer Detection Panel

Based on the colorectal cancer gene mutation panels licensed from the University of Potsdam, 17 mutations in four genes associated with colorectal cancers, APC, BRAF, CTNNB1, and KRAS, are detected in three multiplex qPCR reactions. With the negative, positive, and reference gene controls, the qPCR-amplified mutation targets are used to call out for positive or negative results based on the difference in Cq value between the Cq for mutation target and the Cq for reference gene control.

Utilizing XNA Technology

ColoScape™ assay utilizes our proprietary XNA technology which leverages a sequence-specific clamp made by xeno-nucleic acid (XNA) to suppress PCR amplification of wild-type DNA template and selectively amplify only mutant DNA template. As a result, the sensitivity reaches 0.1% to 0.5% VAF with 10 ng of blood DNA input.

Easy to Operate

The assay can be performed on readily available qPCR instrumentation that is already present in hospital pathology laboratories. Validated instruments include Roche LightCycler® 480 II, Thermo Fisher ABI QuantStudio 5 and Thermo Fisher ABI 7500 Fast Dx.

Streamlined Workflow

1. **DNA Isolation & Quantification**
   Extract DNA from FFPE or plasma using a commercial DNA extraction kit followed by measuring the concentration using fluorometric analysis. Dilute the extracted DNA to the concentration required in the product manual
2. **Preparation of Reagents and Assay Mixes**
   Reconstitute dried-down controls with nuclease-free water. Add samples and reconstituted controls to the assay mixes.
3. **qPCR Setup**
   Transfer sample reagent mixes into PCR plate
   ~ 30 - 60 min
4. **qPCR Programing**
   Enter amplification parameters on qPCR instrument, load PCR plate and start the run
5. **Data Analysis**
   Determine the presence or absence of mutations according to the ΔCq value cutoffs ~ 15 minutes

ANALYTICAL PERFORMANCE

ANALYTICAL PERFORMANCE - ACCURACY

The analytical accuracy is verified and validated through testing of well-characterized samples with known mutations verified by NGS and Sanger sequencing. Studies are conducted to demonstrate concordance in mutation status of FFPE and plasma samples. The results demonstrate a 100% match between reference methods and the ColoScape™ Colorectal Cancer Mutation Detection Test.

ANALYTICAL PERFORMANCE - PRECISION

The precision of the ColoScape™ assay was determined with defined analytical levels of genomic DNA with known mutational status and allelic frequencies. The table on the right shows the summary of reproducibility results of ColoScape™ Colorectal Cancer Mutation Detection Test.

Variation	%CV
Intra-assay	≤ 3%
Inter-assay	≤ 5%
Lot-to-Lot Variation	≤ 4%
Operator Variability	≤ 3%

ANALYTICAL PERFORMANCE - LIMIT OF DETECTION

To determine the limit of detection (LOD) and analytical sensitivity of the kit, mutant allelic frequencies at VAF 1%, 0.5% and 0.1% with 10 ng DNA/reaction were tested.. The LOD of 0.5% is reached for all the mutant targets. Below table shows the LOD summary determined using cfDNA reference standards. **Roche LC 480 II were used*

Target Mutation	Variant Allele Frequency	10ng DNA Input % Correct Call	Target Mutation	Variant Allele Frequency	10ng DNA Input % Correct Call	Target Mutation	Variant Allele Frequency	10ng DNA Input % Correct Call
	1% Mutation	100%		1% Mutation	100%		1% Mutation	100%
APC E1309	0.5% Mutation	100%	APC R1450	0.5% Mutation	100%	KRAS G12	0.5% Mutation	95%
	0.1% Mutation	100%		0.1% Mutation	30%		0.1% Mutation	80%
	1% Mutation	100%		1% Mutation	100%		1% Mutation	100%
APC Q1367	0.5% Mutation	100%	CTNNB1 T41	0.5% Mutation	100%	KRAS G13	0.5% Mutation	100%
	0.1% Mutation	100%		0.1% Mutation	85%		0.1% Mutation	90%
	1% Mutation	100%		1% Mutation	100%		1% Mutation	100%
APC R876	0.5% Mutation	100%	CTNNB1 S45	0.5% Mutation	95%	BRAF V600	0.5% Mutation	100%
	0.1% Mutation	95%		0.1% Mutation	80%		0.1% Mutation	90%

ANALYTICAL PERFORMANCE - SPECIFICITY: CROSS-REACTIVITY

Cross-reactivity of the ColoScape™ assays within the kit was tested with one or more mutations present in a mixed positive control at 5% variant allelic frequency. The data demonstrates that the ColoScape™ Kit can correctly identify several mutations within one panel (one tube). There is cross reactivity between KRAS G12 and KRAS G13, due to the proximity of the mutations, which can be differentiated (Refer to ColoScape™ prodcut manual for more information).

Assay	APC E1309	APC Q1367	APC R1450/876 KRAS G12	CTNNB1 T41	CTNNB1 S45 KRAS G13	KRAS G12	KRAS G13	BRAF V600; CTNNB1 S45; KRAS G13
APC E1309	+	-	-	-	-	-	-	-
APC Q1367	-	+	-	-	-	-	-	-
APC R1450/876	-	-	+	-	-	-	-	-
CTNNB1 T41	-	-	-	+	-	-	-	-
CTNNB1 S45	-	-	-	-	+	-	-	+
KRAS G12	-	-	+	-	*	+	*	*
KRAS G13	-	-	-	-	+	-	+	+
BRAF V600	-	-	-	-	-	-	-	+

Note: "+" indicates mutation detection; "-" indicates no cross-reaction; "" indicates cross-reaction*

DATA FILE

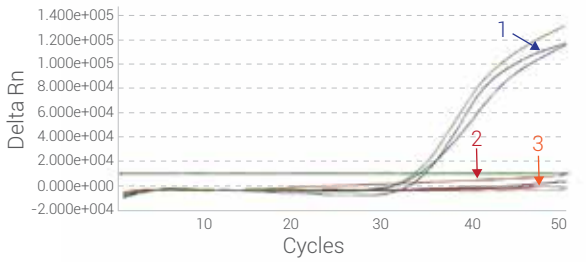


Figure: The APC 1367 Amplification Plot on ABI 7500 Fast Dx showing positive control, negative control and no template control.

Line 1: Positive Control, Cq=34;
Line 2: Negative Control, Cq Undetermined;
Line 3: NTC, Cq Undetermined.

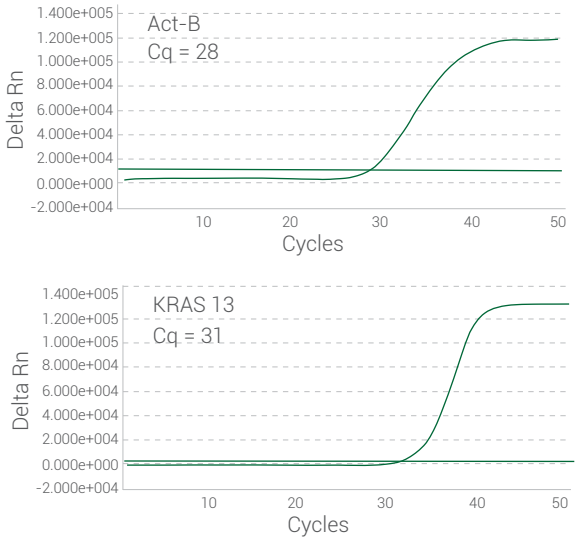


Figure: A run file on ABI 7500 Fast Dx showing a KRAS-positive clinical sample

UTILIZING COLOSCAPE™ ON CLINICAL SAMPLE MUTATION DETECTION

Clinical sensitivity and specificity are tested on the samples extracted from FFPE and plasma of subject with different stages of colorectal cancer from normal to advanced adenomas (pre-cancer), to colorectal cancer stages I to IV. A sample was considered positive if at least one of the target mutations tested positive based on the cutoffs.

The table below shows the summary of clinical sensitivity and specificity for FFPE and plasma patient samples.

Types of Clinical Samples	Patient Number	True Positive	False Positive	True Negative	False Negative	Clinical Parameter	
						Specificity*	Sensitivity*
FFPE	175	138	1	24	12	96.00%	92.00%
cfDNA	119	32	0	83	4	100.00%	88.90%
Pre-cancer AA**	10	6	0	0	3	N/A	66.60%
Pre-cancer cfDNA	58	10	2	40	6	95.20%	62.50%

* Sensitivity = (TP/TP+FN) *100%; Specificity = (TN/TN+FP) *100%
** AA, Advanced Adenomas

FEATURES AND BENEFITS OF COLOSCAPE™ ASSAYS

NON-INVASIVE TESTING

A blood or FFPE samples test intended to diagnose colorectal cancer and occurrence at the early and treatable stage

ULTRA-SENSITIVITY

Detect reliably 0.1% to 0.5% VAF mutant DNA out of wild-type DNA for targeted mutations

CLINICAL SENSITIVITY

66.6% for advanced adenomas (pre-cancer, stage 0); ≥88.9% for colorectal cancer (stage I to IV)

LOW INPUT DNA

10 ng DNA input/reaction needed. Less than one tubes of blood (10mL each) needed for cell-free DNA (cfDNA)

DIAGNOSTIC AID

Aids colonoscopists in the diagnosis of serrated advanced adenomas

IMPORTANT REFERENCE FOR CLINICALLY SIGNIFICANT MUTATIONS

Proprietary XNA technology accurately identifies wild-type and mutant status of clinically relevant genes

FAST RESULT

Less than 4 hours of assay run time

COMPREHENSIVE COVERAGE

Patented gene panel covering 4 genes and 17 mutations

GREAT VERSATILITY

Only requires routine qPCR instruments that are available in common research discovery and pathology labs

PRODUCT SPECIFICATION & ORDERING INFORMATION

Product Name	ColoScape™ Colorectal Cancer Mutation Detection Test (ColoScape Liquid 1.1 Version Kit)		
Pack Size	24 Reactions	**Input DNA**	10 ng/reaction
Catalog # (CE)	N/A	**Instruments Validated**	QuantStudio 5
Catalog # (RUO)	DC-03-0003	**Detection**	TaqMan
Intended Use	Research use only	**Turnaround Time**	Less than 4 hours
Sample Type	FFPE and plasma	**Stability**	Stable for 12 months at 15°C to 25°C (room temperature)

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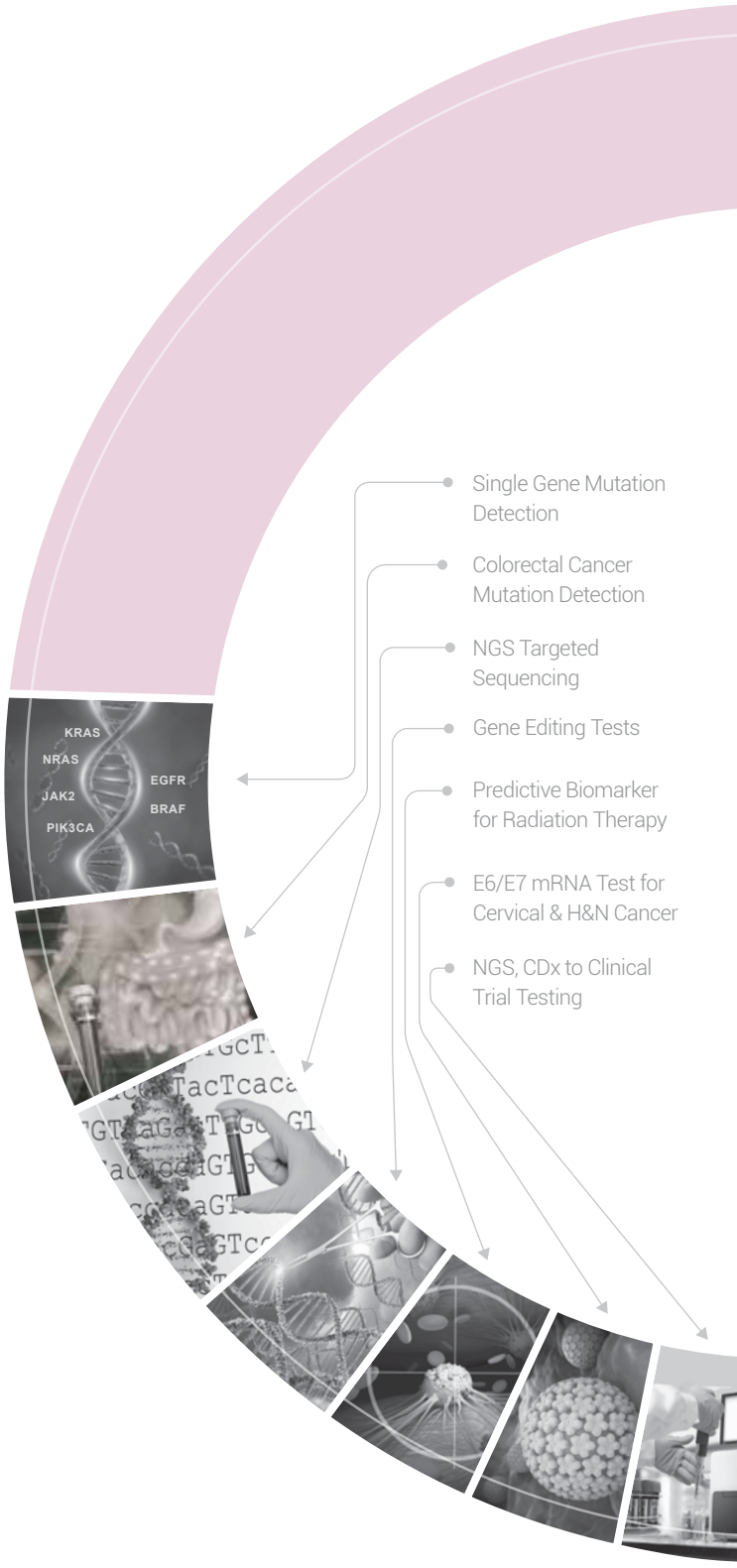
Redefining Precision Molecular Diagnostics through Cancer Gene Mutation Detection



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