DIACARTA

ColoScape™ 2.0 Colorectal Cancer Mutation Detection Test Report

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Patient Details	Specimen Details	Physician Details
Name:	**Specimen Type:**	**Name**
Date of Birth:	**Date collected**	**ID:**
Gender:	**Date Received**	**NPI:**
Patient ID:	**Date Reported**	**Phone**

Patient Sample	Mutation	APC E1309	APC Q1367	APC R1450	CTNNB1 T41	CTNNB1 S45	KRAS G12	KRAS G13	BRAF V600E	TP53 R273H	NRAS G12D	TP53 R175H	TP53 R248Q	PIK3CA E545K	SMAD4 R361C
1	**Positive:**														
	Negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Patient sample	Methylation Marker	FAM			HEX	
1		KCNQ5	C9Orf50	MYO1G	FLI1	CLIP4
	Positive					
	Negative	-	-	-	-	-

Result

ALGORITHIM SCORE	**0**
FINAL RESULT	**GENETIC MUTATION ASSOCIATED WITH INCREASED RISK OF COLORECTAL CANCER IS NOT DETECTED IN THIS TEST PANEL**

These results should be reviewed by the physician to obtain further health care advice and develop a personalized treatment plan. The physician can use this information to customize care, which could include increased screening, preventative surgery, medication, and other steps.

TEST METHODOLOGY

ColoScape™ 2.0 Colorectal Cancer Mutation Detection Test is a multiplex qPCR-based *in vitro* diagnostic assay for qualitative detection of colorectal cancer-associated mutations in genes. The test is based on DiaCarta's Xenonucleic acid (XNA) mediated molecular clamping technology and uses the colorectal cancer (CRC) gene panel licensed from Dr. Bettina Scholtka from University of Potsdam, Germany. [1, 2]

The assay uses XNA clamps to specifically bind to and block the wild-type (normal) target gene sequence and selectively amplify the mutant DNA target sequence. From the DNA samples extracted from FFPE (Formalin-fixed paraffin embedded) or plasma, and the detection kit identifies the presence or absence of mutations in the targeted regions on APC, CTNNB1, KRAS, NRAS,TP53, PIK3CA, SMAD4 or BRAF genes. Within three reactions, 8 genes and 25 mutations can be detected within 4 hours. The ColoScape™ 2.0 Colorectal Cancer Mutation Detection Test enables the detection of low frequency gene mutations within the background of normal (wild type) DNA.

CLINICAL RELEVANCE OF GENE VARIANTS IN THE PANEL

Normal cells can turn into cancer cells largely because of mutations in their genes. The mutations may affect different genes that control cell growth and division. As mutations accumulate, the cells become more abnormal, and cancer progresses. The following genes play a critical role in the development of colorectal cancer:

Gene	Function	Types of Cancer	Incidence in CRC	Effect on Drug Sensitivity	Effect on Drug Resistance
APC	Adenomatous **p**olyposis **c**oli (APC) is a tumor suppressor gene that plays a role in cell signaling. [3]	Colorectal adenocarcinoma, Prostate adenocarcinoma, Colon adenocarcinoma, Lung adenocarcinoma, and Cancer of unknown primary [4] Fmilial adenomatous polyposis (FAP)	**47.00%** colorectal carcinoma patients. [8]	FOLFIRI+Bevacizumab has recently also been associated with complete remission [9]	5-FU resistance [10]
CTNNB1	CTNNB causes activation of Wnt signal pathway that causes unregulated growth of cells. [5]	Colon adenocarcinoma, Endometrial endometrioid adenocarcinoma, Lung adenocarcinoma, Prostate adenocarcinoma, and Hepatocellular carcinoma. [4] Colorectal cancers with microsatellite instability (MSI)	**4.81%** of colorectal carcinoma patients. [8]	• Vantictumab • Ipafricept • SFRP peptide • PORCN inhibitor. [11]	Cetuximab, Oxaliplatin, irinotecan, SN-38 and 5-FU resistance. [12]

Gene	Function	Types of Cancer	Incidence in CRC	Effect on Drug Sensitivity	Effect on Drug Resistance
BRAF	BRAF regulates the MAP kinase signaling pathways affecting cellular proliferation and differentiation. [6]	Colon adenocarcinoma, Thyroid gland papillary carcinoma, Cutaneous melanoma, Melanoma, and Lung adenocarcinoma [4] Associated with serrated adenomas, mainly in the right colon, in women	**12%** of colorectal carcinoma patients. [8]	• Cetuximab, encorafenib, and binimetinib have evidence of efficacy.[13] • Cetuximab and irinotecan with or without vemurafenib show improved response rate [14]	Resistance to anti-EGFR therapy can be seen. [15]
KRAS	KRAS gene is involved in kinase signaling pathways *RAS* overexpression can lead to continuous cell proliferation. [7]	Colorectal adenocarcinoma, Lung adenocarcinoma, Colon adenocarcinoma, Endometrial endometrioid adenocarcinoma [4] Codon 12 is associated with mucinous colorectal CA, codon 13 mutation with non-mucinous cancers	**32%** of colorectal carcinoma patients.[8]	FDA Approved: ERBITUX(R) (cetuximab) + FOLFIRI (Irinotecan, 5-Fluorouracil, Leucovorin) [16]	Wild-type *RAS,* mCRC can show resistance to anti-EGFR therapy . [16]
TP53 R273 H	P53-R273H mutation enhances colorectal cancer stemness through regulating specific lncRNAs [17] It represents one of the best characterized tumor suppressor genes and is located on the short arm of chromosome 17 (17p13.1)	TP53 R273H is present in cases with colon adenocarcinoma, breast invasive ductal carcinoma, colorectal adenocarcinoma, pancreatic adenocarcinoma, and rectal adenocarcinoma having the greatest prevalence [4]	**60%** of colorectal carcinoma patients [17]	The addition of VEGF- and EGFR-targeted antibodies to standard chemotherapeutic regimens benefits the CRC patients. Also, tyrosine kinase inhibitors like Regorafenib is found effective in p53 mutated tumors. [18]	It is currently assumed that CRC cell lines lacking functional p53 are more resistant to 5-FU and Oxaliplatin [19]

Gene	Function	Types of Cancer	Incidence in CRC	Effect on Drug Sensitivity	Effect on Drug Resistance
TP53 R175H	The p53 mutant R175H and R273H are found to promote invasive behavior in cancer and normal cells in vitro and in vivo. This is mediated by AKT signaling activation driven by p53 mutants. [17]	TP53 R175H is present in 1.95% of AACR GENIE cases, with colon adenocarcinoma, pancreatic adenocarcinoma, colorectal adenocarcinoma, breast invasive ductal carcinoma, and rectal adenocarcinoma having the greatest prevalence. [4]	**60%** of colorectal carcinoma patients [17]	The addition of VEGF- and EGFR-targeted antibodies to standard chemotherapeutic regimens benefits the CRC patients. Also, tyrosine kinase inhibitors like Regorafenib are found effective in p53 mutated tumors. [18]	It is currently assumed that CRC cell lines lacking functional p53 are more resistant to 5-FU and Oxaliplatin [19]
TP53 R248Q	R248Q has a greater tendency to aggregate and can seed the aggregation of wt p53. most common p53 mutant R248Q (mutp53) enhances Stat3 activation. [20]	TP53 R248Q is present in 1.27% of AACR GENIE cases, with colon adenocarcinoma, breast invasive ductal carcinoma, lung adenocarcinoma, colorectal adenocarcinoma, and pancreatic adenocarcinoma having the greatest prevalence [4].	**60%** of colorectal carcinoma patients. [17]	Combination therapy of SIRT inhibitors and first-line chemotherapeutic drugs may be beneficial for the treatment of patients with *TP53*^{mut} CRC. [21]	wild-type (wt) *TP53*, SIRT inhibitors were found to act antagonistically with multiple chemotherapeutic agents (cisplatin, 5-fluorouracil, oxaliplatin, gefitinib, LY294002 and metformin), and decreased the anti-tumor effects of these agents. [21]
NRAS G12D	The NRAS G12D mutation arises from a single nucleotide change (c.35G>A) and results in an amino acid substitution of the glycine (G) at position 12 by an aspartic acid (D). [22]	NRAS G12D is present in 0.37% of AACR GENIE cases, with acute myeloid leukemia, colon adenocarcinoma, colorectal adenocarcinoma, rectal adenocarcinoma, and acute myeloid leukemia with myelodysplasia-related changes having the greatest prevalence [4].	**4%** of colorectal carcinoma patients [8]	Improvements in overall survival were observed with panitumumab–FOLFOX4 therapy. [23]	NRAS has primary and acquired resistance of CRC to anti-Epidermal Growth Factor Receptor (EGFR) antibodies therefore associated with resistance to anti-EGFR therapy. [22]

Gene	Function	Types of Cancer	Incidence in CRC	Effect on Drug Sensitivity	Effect on Drug Resistance
SMAD4 R361C	SMAD4 encodes a tumor suppressor and transcription factor that is a downstream effector in the TGF-ss signal transduction pathway. SMAD4 mutations in colorectal cancer probably occur before chromosomal instability, but after divergence of the microsatellite instability pathway	SMAD4 R361C is present in 0.26% of AACR GENIE cases, with colon adenocarcinoma, colorectal adenocarcinoma, pancreatic adenocarcinoma, lung adenocarcinoma, and rectal adenocarcinoma having the greatest prevalence [4].	**10%** of colorectal carcinoma patients [8]	Loss of SMAD4 and deletion of chromosome 18q have been extensively elaborated and shown to correlate with colorectal metastasis, resistance to 5-fluorouracil chemotherapy and poor outcome. [24]	Personalized treatment therapies with combination chemotherapy. [24]
PIK3CA E545K	(PIK3CA) is one of the most mutated genes in CRC. *PIK3CA* encodes the p110 catalytic subunit of PI3K, which is one of the crucial kinases in PI3K/Akt/mTOR signaling. [25]	PIK3CA E545K is present in 2.61% of AACR GENIE cases, with breast invasive ductal carcinoma, colon adenocarcinoma, lung adenocarcinoma, bladder urothelial carcinoma, and breast invasive lobular carcinoma having the greatest prevalence [4].	**14%** of colorectal carcinoma patients [8]	Delalisib has been approved by the US Food and Drug Administration as the first PI3K inhibitor in cancer treatment. There are also multiple agents targeting PIK3CA in development Moreover, collective reports suggested that PIK3CA mutated CRC patients will benefit from Aspirin administration and radioembolization than wild type ones. [26]	PIK3CA Mutations Contribute to Acquired Cetuximab Resistance in Patients with Metastatic Colorectal Cancer. [26]

CLINICAL SENSITIVITY AND SPECIFICITY OF COLOSCAPE™ 2.0

A clinical trial performed using a novel Xenonucleic acid mediated molecular clamping technology in 380 clinical samples including both plasma cfDNA and tissue FFPE samples from patients with precancerous state and different stages of CRC to determine clinical performance. [17]

	Sensitivity	Specificity
Precancerous Mutation Detection	**62 to 66.6%**	**95.2%**
Colorectal Cancer Mutation Detection	**92%**	**96% to 100%**

RECOMMENDATIONS BY AMERICAN CANCER SOCIETY

Current American Cancer Society [18] guidelines emphasize additional screening such as virtual colonoscopy every 5 years beginning at age 45, and colonoscopy every 10 years. Those who are at high risk or have polyps are recommended to do colonoscopy every 3 years.

DISCLAIMER

The Coloscape™ 2.0 Colorectal Cancer Mutation Test developed at DiaCarta uses the gene panel licensed from University of Potsdam (Germany). This gene panel has not been approved by the US FDA as an IVD and is available as a Research Use Only test. It has been validated as a lab developed test (LDT) at DiaCarta's CLIA Certified Clinical Laboratory.

The assay is designed to perform on DNA extracted from Plasma or FFPE (Formalin-fixed paraffin embedded). The test identifies the presence or absence of mutations in the targeted region.

Cancer is a heterogeneous disease that can occur because of somatic mutations in various driver genes. Therefore, this test should not be interpreted solely without the use of other assessment tools, including personal and family history, imaging studies, and/or other laboratory analyses. Literature as referenced above mentions that the presence of gene mutations indicates an increased risk of colorectal cancer, and this result should be interpreted along with other clinical findings. Absence of gene mutation in the sample does not eliminate the risk of colorectal cancer as there can be involvement of 1 or more of the gene variants on the panel that is not identified by the test method.

For questions about this report or to speak with the DiaCarta oncology support team, please call +1 (800) 878-6662.

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