ColoScape™ Test: A Molecular Assay to Detect Early-Stage Colorectal Cancer in Plasma Cell-Free DNA

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INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer deaths when men and women are combined in the U.S. Early detection in the precancerous stage is key to reducing the CRC morbidity and mortality rates. Thus, there is a critical need for a cost-effective, time-efficient, and convenient clinical tool for early CRC detection.

We have developed a multiplex qPCR assay (ColoScape™) to detect CRC-associated genetic and epigenetic changes from liquid biopsy samples (i.e., cell-free DNA) using our proprietary QClamp® XNA technology. The QClamp® XNA technology is very unique and enables the ColoScape™ assay to selectively amplify the mutant and methylated DNA target sequences by using a synthetic DNA analog XNA (Xenonucleic Acid) (Figs 1 & 2).

This study introduces our new ColoScape™ test for detecting CRC-associated mutations and methylation markers in plasma cell-free DNA (cfDNA).

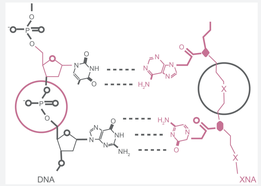


Figure 1. XNA structure and hybridization with DNA

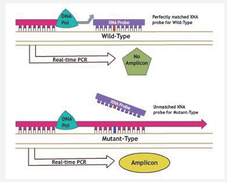
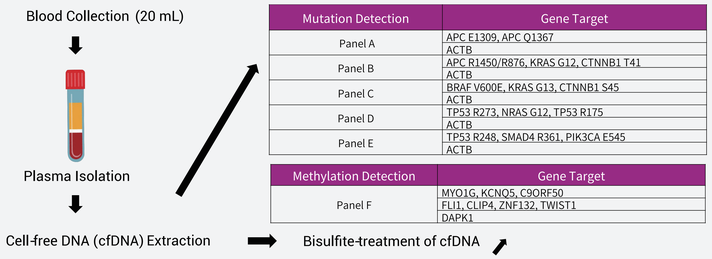


Figure 2. Principle of the ColoScape™ mutation and methylation detection in targeted genes.

MATERIALS AND METHODS

The Coloscape™ test is designed to detect mutations in 8 genes, 61 mutations and 7 methylated markers (Fig 3). The test consists of two parts: (i) the detection of mutations in 8 genes (APC, KRAS, BRAF, TP53, CTNNB1, NRAS, SMAD4, and PIK3CA) and (ii) the detection of 7 methylation targeted genes. We designed PCR primers, TaqMan probes, and XNA in each mutation and methylation gene target for multiplexing. The assay analytical performance was verified and validated using various control samples. Furthermore, we have conducted a case-control pilot study in comparison with the clinical status to evaluate the clinical sensitivity and specificity of the ColoScape™ test.



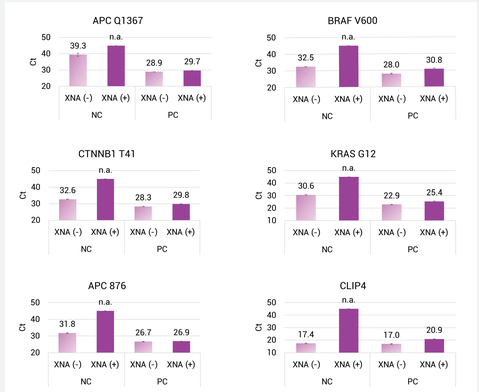
Mutation Detection	Gene Target
Panel A	APC E1309, APC Q1367
	ACTB
Panel B	APC R1450/R876, KRAS G12, CTNNB1 T41
	ACTB
Panel C	BRAF V600E, KRAS G13, CTNNB1 S45
	ACTB
Panel D	TP53 R273, NRAS G12, TP53 R175
	ACTB
Panel E	TP53 R248, SMAD4 R361, PIK3CA E545
	ACTB

Methylation Detection	Gene Target
Panel F	MYO1G, KCNQ5, C9ORF50
	FLI1, CLIP4, ZNF132, TWIST1
	DAPK1

Figure 3. Assay Workflow

RESULTS

1. Assay Performance



NC, Negative Control (wildtype DNA); PC, Positive Control (5% VAF); n.a., no amplification

Figure 4. Assay Feasibility: XNA effectively suppressed wild-type allele amplification.

Table 1. Limit of Blank (LoB): Clinical LoB was determined using cfDNA samples from healthy donor plasma. n.a., no amplification

Target	Analytical LoB (Ct)	Clinical LoB (Ct)	Target	Analytical LoB (Ct)	Clinical LoB (Ct)	Target	Analytical LoB (Ct)	Clinical LoB (Ct)
APC E1309	n.a.	n.a.	BRAF V600	n.a.	n.a.	TP53 R273	44.6	40.4
APC Q1367	n.a.	n.a.	CTNNB1 S45	44.4	40.7	PIK3CA E545	43.7	40.0
APC R1450/R876	40.2	39.2	KRAS G13	43.4	42.4	SMAD4 R361	n.a.	n.a.
CTNNB1 T41	41.7	41.3	NRAS G12	43.0	38.6	TP53 R248	41.8	41.1
KRAS G12	42.5	41.9	TP53 R175	42.7	36.6	Methylation Panel 1 & 2	n.a.	n.a.

Table 2. Analytical Limit of Detection (LoD): cfDNA reference standards were used.

Target	VAF% (Mutation or Methylation)	% Correct Call (10 ng Input cfDNA)	Target	VAF% (Mutation or Methylation)	% Correct Call (10 ng Input cfDNA)
APC E1309	1%	100%	NRAS G12	1%	100%
	0.50%	100%		0.50%	100%
	0.10%	65%		0.10%	95%
APC Q1367	1%	100%	TP53 R175	1%	100%
	0.50%	100%		0.50%	100%
	0.10%	100%		0.10%	100%
APC R1450/R876	1%	100%	TP53 R273	1%	100%
	0.50%	100%		0.50%	100%
	0.10%	90%		0.10%	100%
CTNNB1 T41	1%	100%	PIK3CA E545	1%	100%
	0.50%	100%		0.50%	100%
	0.10%	100%		0.10%	90%
CTNNB1 S45	1%	100%	SMAD4 R361	1%	100%
	0.50%	100%		0.50%	100%
	0.10%	90%		0.10%	100%
KRAS G12	1%	100%	TP53 R248	1%	95%
	0.50%	100%		0.50%	95%
	0.10%	100%		0.10%	100%
KRAS G13	1%	100%	Methylation Panel1	1%	95%
	0.50%	100%		0.50%	95%
	0.10%	90%	Methylation Panel2	1%	100%
BRAF V600	1%	100%		0.50%	100%
	0.50%	100%			
	0.10%	100%			

2. Clinical Performance

Table 3. 2X2 Contingency Tables: The two pilot studies were performed using 77 subjects and 38 subjects, which the clinical status was known.

CRC		Clinical Status		Sensitivity (%)	Specificity (%)
		Positive	Negative		
ColoScape™ Test	Positive	24	0	85.7 % (95% CI: 0.66-0.95)	100% (95% CI: 0.91-1.00)
	Negative	4	49		
	Total	28	49		

AA		Clinical Status		Sensitivity (%)	Specificity (%)
		Positive	Negative		
ColoScape™ Test	Positive	3	3	60% (95% CI: 0.17-0.93)	91 % (95%CI: 0.75-0.98)
	Negative	2	30		
	Total	5	33		

Table 4. Mutation Status in advanced adenoma samples. The ColoScape™ test identified APC or TP53 mutation in AA.

Specimen	Mutation Status
AA #022	APC
AA #079	APC
AA #197	TP53
AA #188	Undetected
AA #137	Undetected

CONCLUSIONS

1. Assay Performance

- **LoB/LoD:** 0.5% variant allelic frequency and 0.5% methylation can be detected with 10 ng DNA input.
- **Precision:** The ColoScape™ test showed high inter, intra, lot-to-lot and operator reproducibility with CV≤10% and has good reproducibility with instrument comparison with CV≤10%.
- **Specificity/Cross-reactivity :** No adverse cross-reactivity was observed.
- **Matrix Interference:** No adverse effect on the assay performance was observed with up to 10% Ethanol spiked.

2. Clinical Performance: The preliminary assay clinical specificity and sensitivity were 100% (95% CI: 91.3-100%) and 86% (95% CI: 66-95%) respectively for CRC and 91% specificity (95% CI: 75%-98%) and 60% sensitivity (95% CI: 17%-93%) for advanced adenomas.

In summary, the ColoScape™ test utilizing the QClamp® XNA-based technology provides high sensitivity and high specificity to advanced adenomas in addition to CRC with a great potential to be used as an early screening test.

REFERENCES

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