Effect of blood collection tubes on circulating tumor DNA (ctDNA) yield and specificity

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Introduction

Solid tumors, such as colorectal cancer (CRC), may leak DNA (circulating tumor-DNA, ctDNA) into blood. ctDNA can be detected by assaying circulating cell free DNA (ccfDNA) isolated from plasma for tumor-specific features such as methylated *BCAT1* and *IKZF1*. Accurate identification of ctDNA relies on minimum release of genomic DNA such as from lysed white blood cells during blood collection, transport and processing. The aim of this study was to evaluate a ccfDNA collection tube and extraction chemistry in a cohort of patients with and without CRC.

Methods

Blood was collected in K_3EDTA and PAXgene Blood ccfDNA tubes (Qiagen) from 79 colonoscopy confirmed subjects that were over 50 years of age. Plasma was isolated by centrifugation within 8 hours of K_3EDTA blood collection, whereas the PAXgene tubes were stored at room temperature for 7 days prior to plasma isolation. ccfDNA was extracted from 4mL of K_3EDTA blood tube plasma using the QIAsymphony (QS) DSP Virus/Pathogen kit and 4.8mL of PAXgene blood ccfDNA tube plasma using the QS PAXgene Blood ccfDNA extraction kits. Extracted DNA was bisulphite converted using the Epitect Fast Bisulphite conversion kit (Qiagen). Successful recovery of ccfDNA was measured simultaneously (*ACTB*) with detection of ctDNA by assaying for the presence of methylated *BCAT1* and *IKZF1* DNA. Qualitative results were determined using second derivative cycle threshold (Ct) results. Quantitative results were determined using fit point Ct values (fluorescence signal threshold set as 2 and 3 for *IKZF1* and *BCAT1* respectively) and a standard curve generated using an ordinary least squared regression. Results were reported as methylated DNA (*BCAT1+IKZF1*) per mL of plasma input.

Figure 1 I Study Design

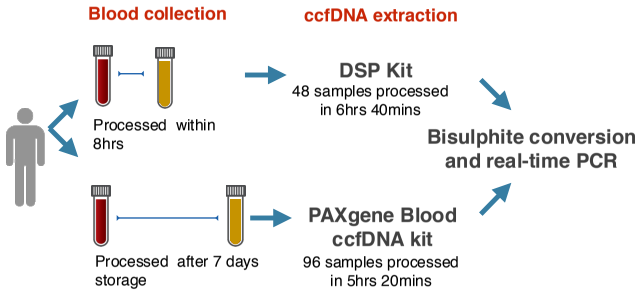
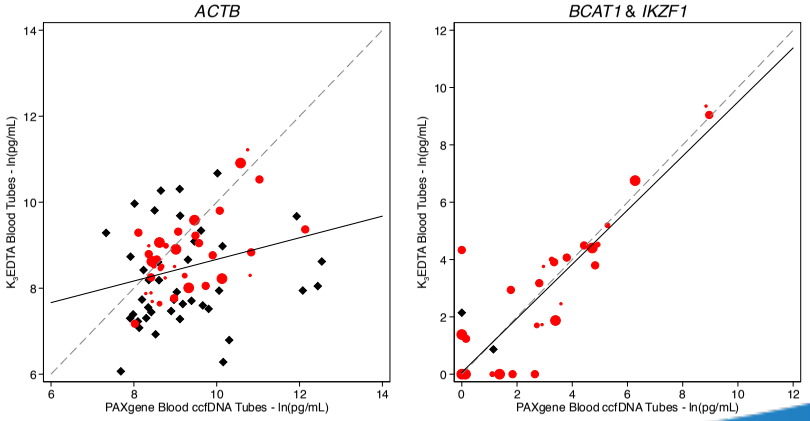


Table 1 I Study cohort and ctDNA Results

Gene	Phenotype/ Stage	n	K3EDTA % Pos	K3EDTA 95% CI	PAXgene % Pos	PAXgene 95% CI
BCAT1	All Cancer	38	52.6	(35.8-69.0)	52.6	(35.8-69.0)
	Stage I	9	44.4	(13.7-78.8)	44.4	(13.7-78.8)
	Stage II	7	42.9	(9.9-81.6)	57.1	(18.4-90.1)
	Stage III	15	60.0	(32.3-83.7)	46.7	(21.3-73.4)
	Unstaged	7	57.1	(18.4-90.1)	71.4	(29.0-96.3)
	Non-Cancer[1]	41	7.3	(1.5-19.9)	2.4	(0.1-12.9)
IKZF1	All Cancer	38	42.1	(26.3-59.2)	42.1	(26.3-59.2)
	Stage I	9	55.6	(21.2-86.3)	44.4	(13.7-78.8)
	Stage II	7	42.9	(9.9-81.6)	42.9	(9.9-81.6)
	Stage III	15	40.0	(16.3-67.7)	46.7	(21.3-73.4)
	Unstaged	7	28.6	(3.7-71.0)	28.6	(3.7-71.0)
	Non-Cancer[1]	41	7.3	(1.5-19.9)	4.9	(0.6-16.5)
Combined	All Cancer	38	57.9	(40.8-73.7)	63.2	(46.0-78.2)
	Stage I	9	55.6	(21.2-86.3)	44.4	(13.7-78.8)
	Stage II	7	57.1	(18.4-90.1)	71.4	(29.0-96.3)
	Stage III	15	60.0	(32.3-83.7)	66.7	(38.4-88.2)
	Unstaged	7	57.1	(18.4-90.1)	71.4	(29.0-96.3)
	Non-Cancer[1]	41	14.6	(5.6-29.2)	4.9	(0.6-16.5)

[1]Includes 40 non-neoplastic cases and 1 case with a Peutz–Jeghers Polyp.

Figure 2 I Correlation graphs of total yield of DNA (left) and ctDNA (right) obtained by the two systems tested



RESULTS

The two systems were evaluated in 38 cases with colorectal cancer, 40 cases with no evidence of disease and 1 case with a Peutz–Jeghers Polyp. There was no significant difference in the positivity rates between the two systems for CRC or non-neoplastic cases and good concordance was observed between the two systems (Non-neoplastic, 85%: CRC, 80%), Table 2.

A higher yield of total DNA (*ACTB*) was measured in the PAXgene system (Median ng/mL (IQR): PAXGene, 7.9 (4.5–16.9); K3EDTA, 4.5 (2.2–8.8), p value=0.005). Despite the higher total DNA no increase in the positivity rates in the non-neoplastic cases was observed (5% and 15% for the PAXGene and K3EDTA systems, respectively). Yield correlation plots for total DNA and tumor DNA (*BCAT1* and *IKZF1*) are shown in Figure 2. The total amount of DNA recovered between the two systems showed poor correlation (rho=0.27) whereas the ctDNA yields correlated well (rho=0.92).

Table 2 I Contingency tables for method comparison

Non-neoplastic		PAXgene +	PAXgene -
K_3EDTA	+	0	5
	-	1	34
McNemar's p value:		0.1025	

CRC		PAXgene +	PAXgene -
K_3EDTA	+	19	3
	-	5	11
McNemar's p value:		0.4795	

CONCLUSION

Storing whole blood for 7 days in the new PAXgene Blood ccfDNA collection tube has no downstream effect on accurate detection of methylated *BCAT1/IKZF1* ctDNA in CRC patients. The QS PAXgene Blood ccfDNA extraction kit doubles the extraction throughput and reduces the instrument time by 1hr 20mins. The PAXgene Blood ccfDNA system provides a solution for maintaining preanalytical sample integrity prior to ctDNA testing.

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