Cell-Free DNA BCT® is Compatible with Bisulfite Conversion and Methylation-Specific qPCR in the Epi proColon Assay

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BACKGROUND

Detection of cancer-associated methylation in cell-free DNA (cfDNA) has emerged as a promising approach for liquid biopsy testing.

However, white blood cell lysis and subsequent release of genomic DNA in liquid biopsy samples require that cumbersome pre-analytical steps be taken to ensure reliable and accurate test results.

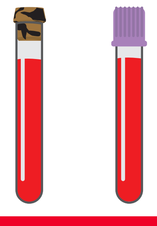
Streck's Cell-Free DNA BCT is a blood collection device that is well-documented to prevent the release of genomic DNA into plasma by stabilizing white blood cells, allowing convenient sample collection, storage, and transport.

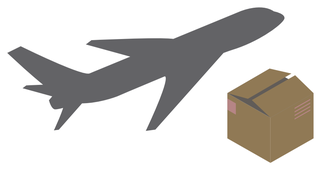
In the present study, we sought to investigate the compatibility of our stabilization technology with bisulfite conversion and methylation-specific PCR of cfDNA.

CELL-FREE DNA BCT

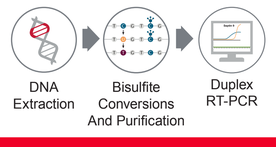
- Stabilizing reagent that preserves the separation of the cell-free and cellular DNA fractions
- Easy sample collection at hospitals and medical practitioner's offices
- Room temperature shipping to the testing lab
- Ensures consistency of patient material and reliability of test results

EXPERIMENTAL METHODS





epi pro colon®



Proteinase K

Blood draws from healthy donors and colorectal cancer patients were collected in Cell-Free DNA BCTs or EDTA tubes

Samples subjected to various room temperature storage and shipping conditions

Samples were then analyzed using the Epi proColon® assay to detect the presence of methylated Septin9 (mSEPT9) DNA

Where noted, a modified lysis step to include proteinase K treatment for 1 hour at 60 °C was included

STUDY 1: DETECTION OF METHYLATED DNA SPIKED INTO BLOOD FROM HEALTHY DONORS

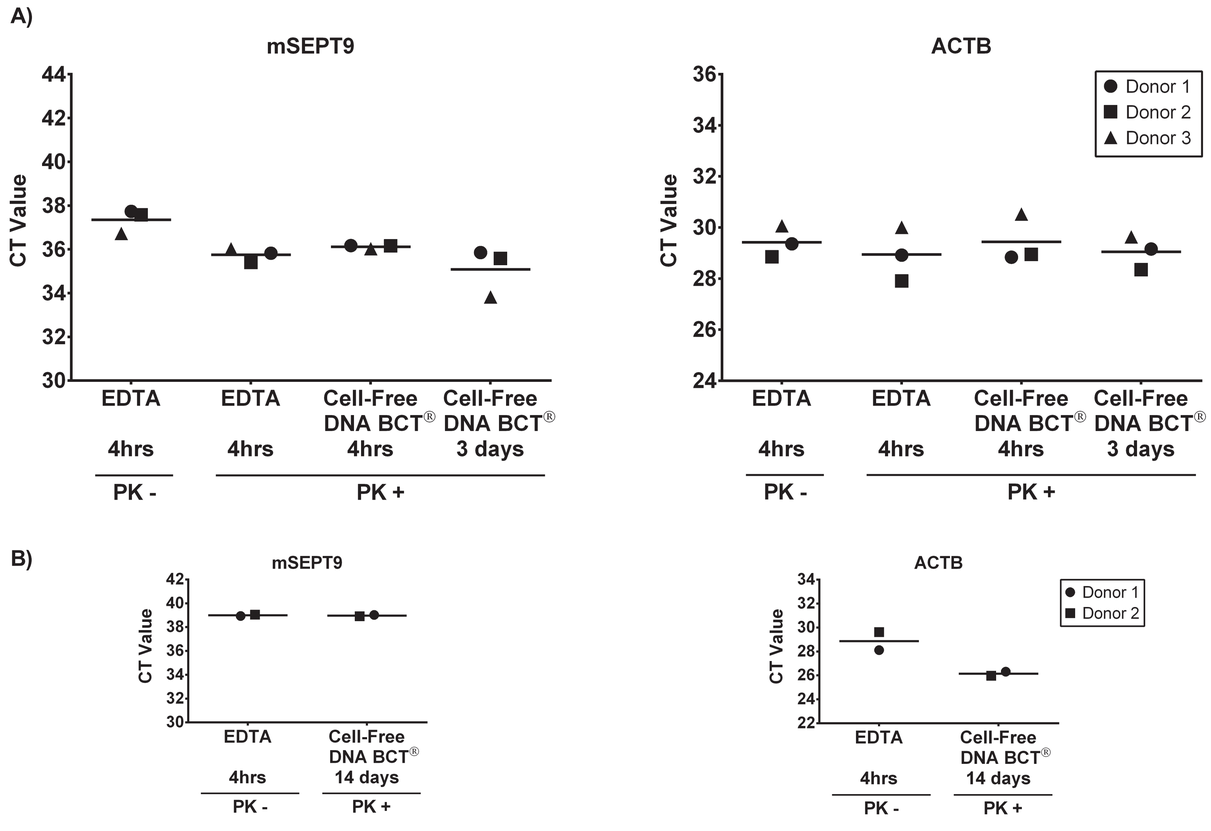


Figure 1:
Whole blood was collected from healthy donors into Cell-Free DNA BCTs and EDTA tubes. Blood was spiked with genomic DNA containing methylated Septin9 for a final concentration of (A) 35pg/mL or (B) 5pg/mL. After the indicated storage times, plasma was separated and analyzed using the Epi proColon test. Where noted, a proteinase K treatment was added to the lysis step. Assay CT values are depicted for each donor, and the black bar designates mean CT.

STUDY 2: FALSE POSITIVE DETECTION IN NON-SPIKED BLOOD SAMPLES

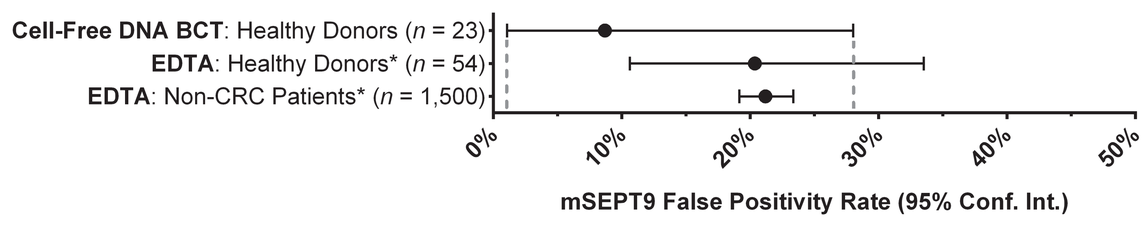


Figure 2:
Whole blood was collected from four healthy donors into Cell-Free DNA BCTs. After three days of room temperature storage, plasma from all donors was separated and pooled. Twenty-three plasma pool replicates were analyzed using the Epi proColon test with a proteinase K step added. False positivity rate with 95% confidence interval was calculated. *Data for EDTA samples obtained from Potter N et. al. 2014. "Validation of a real-time PCR-based qualitative assay for the detection of methylated SEPT9 DNA in human plasma." *Clinical Chemistry.* 60(9):1183-1191. PMID: 24938752.

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STUDY 3: DETECTION OF ENDOGENOUS METHYLATED DNA IN COLORECTAL CANCER PATIENT SAMPLES

Treatment	Collection Tube	Post-Phlebotomy Storage Time	Shipping	PK+ Lysis
A	EDTA	4 hours	Frozen Plasma	No
B	Cell-Free DNA BCT	4 hours	Frozen Plasma	Yes
C	Cell-Free DNA BCT	3 days	Room Temperature Whole Blood	Yes

mSEPT9 Qualitative Results

Donor	Treatment A	Treatment B	Treatment C
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	-	-	-
6	+	+	+

ACTB Qualitative Results

Donor	Treatment A	Treatment B	Treatment C
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+

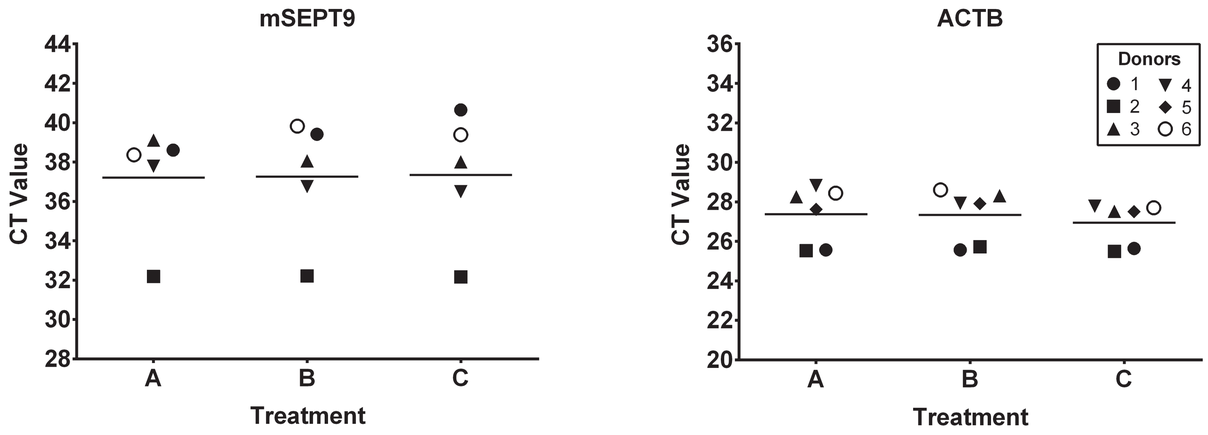

Figure 3:
Whole blood was collected from late stage colorectal cancer patients into Cell-Free DNA BCTs and EDTA tubes. Plasma was separated at the collection facility within 4 hours of phlebotomy and shipped frozen to Streck (treatments A and B) or whole blood was shipped at room temperature to Streck and plasma was separated 3 days post-phlebotomy (treatment C). All samples were analyzed using the Epi proColon test. Where noted, a proteinase K treatment was added to the lysis step. Assay CT values for Sept9 and ACTB are depicted for each donor, and the black bar designates mean CT.

RESULTS

Storage and shipment of whole blood in the Cell-Free DNA BCT did not alter the detection of methylated Septin9 DNA spiked into healthy donor samples or endogenously present in CRC patient samples.

Storage of blood in the Cell-Free DNA BCT did not induce false positive detection of methylated DNA.

Addition of proteinase K treatment did not hinder Epi proColon assay performance.

CONCLUSION

The present study demonstrates that Streck's Cell-Free DNA BCT can be used for cfDNA tests employing bisulfite conversion. This allows the logistical improvements afforded by the Cell-Free DNA BCT to be utilized in methylation-based liquid biopsy tests.