Analytical Validation of a Circulating Tumor Methylated-DNA Assay for Detection of Colorectal Cancer Recurrence

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INTRODUCTION

ColVERa™ is a qualitative epigenetic assay that detects circulating tumor DNA by measuring the methylation within two genes: BCAT1 (Branch chain amino acid transaminase 1) and IKZF1 (Ikaros Family zinc finger 1) that have been determined to be associated with colorectal cancer recurrence. Previous publications have described the assay’s development [1], analytical validation [2] and clinical [3,4,5] validations in an EDTA-based system. We report here the analytical validation of ColVERa in EDTA-based system and in a PAXgene based system as an LDT in a US CLIA licensed laboratory. PAXgene system allows specimen transport flexibility for up to 7 days at ambient temperature.

RESULTS

ColVERa TEST DESCRIPTION

Blood is collected either in 1) two EDTA tubes, processed to plasma within 7 hrs of collection & shipped frozen or 2) two PAXgene Blood cfDNA tubes (QIAGEN; Cat. No. 768536) & 1.5 mL additive & 10 mL blood draw) that are shipped to RT within 7 days from which plasma is generated in the lab. DNA is extracted on a QIAasympo SP using the QIAasympo DSP Virus/Pathogen kit (QIAGEN; Cat. No. 937055) from 4mL EDTA plasma & QIAasympo PAXgene Blood cfDNA Kit (QIAGEN; Cat. No. 768536) from 4mL PAXgene plasma. Extracted DNA is spiked with human cfDNA derived from non-cancerous individuals, and run on the QIAasympo Fast 96 Bisulfite Conversion kit (QIAGEN; Cat. No. 95720) on QIAcube HT. Bis-DNA is measured in triplicate by realtime triplex PCR on LightCycler 480 II using custom methylation specific primers & probes (IDT) and QuantiTect Multiplex PCR No ROX Mastermix (QIAGEN; Cat. No. 204743). ColVERa is called positive when ACTB [β-actin] is positive and any replicate of BCAT1 or IKZF1 has a positive C1.

MATERIALS AND METHODS

Blood from self-declared normal individuals, collected in EDTA or PAXgene blood tubes was processed to plasma within 7 hrs of draw and stored < -20°C unless being used for PAXgene stability & accuracy.

Accuracy & analytical sensitivity: pooled plasma from self-declared normal donors spiked with methylated human gDNA (mDNA) at different concs. (1.6 – 250 pg/mL).

Interfering substances: pooled plasma (EDTA) or blood (PAXgene) with 50pg/mL mDNA, mixed with commonly found blood components at their upper reference ranges.

Accuracy (EDTA): split sample analysis with Clinical Genomics Lab in New Jersey (NJ) & Australia (AUS) using 20 donors (un-spiked and spiked at 5 different mDNA conc.)

Accuracy (PAXgene): donor (colonoscopy confirmed 20 normal & 25 CRC) blood collected in 2 EDTA (plasma within 7hrs) and 2 PAXgene tubes (plasma after 7 days at RT). Stability (PAXgene): pooled blood from self-declared normal individuals (un-spiked and 50pg/mL mDNA) stored at RT for 0, 5 & 8 days before processing. All samples were processed as the Blood Test described above.

LoD: The LoD for ColVERa in EDTA system is 17.1pg/mL (ln = 2.84) and in PAXgene system is 7.5pg/mL (ln=2.01).

Precision: The test has excellent reproducibility for samples above its LoD.

Interfering Substances:

Following did not interfere with the assay:

- Both validation studies: cholesterol (5 mg/mL), EDTA (20 mg/mL), genomic DNA (100 ng/mL), glucose (10 mg/mL), hemoglobin (1 mg/mL), BIC (0.4% v/v), triglycerides (12 mg/mL) & uric acid (0.24 mg/mL).
- PAXgene validation: albumin (40mg/mL), bilirubin (0.2 mg/mL).
- EDTA validation: EDTA (20mg/mL).

Following interfering with the assay:

- EDTA validation: albumin (40 mg/mL) & bilirubin (0.2 mg/mL); 90% concordance with control samples.
- PAXgene validation: When additive to blood ratio is higher than recommended, (1.5 : 10), it negatively affects ColVERa results. Less than 9ml blood volume is not acceptable for testing.

Accuracy:

The test’s accuracy is comparable between sites and between the two tube types.

Precision:

The test has excellent reproducibility for samples above its LoD.

Stability in PAXgene cfDNA tubes:

Amount of ACTB increased over time, likely due to WBC lysis, but that had no impact on ColVERa results.

ColVERa comparison with CEA: ColVERa clinical sensitivity is twice that of CEA (61.5% vs 30.7%) and specificity is 85% & 100% respectively when tested in colonoscopy confirmed 20 normal and 26 CRC specimens.

CONCLUSION

ColVERa assay was successfully validated in a CLIA-licensed clinical lab (#312122075) as a LDT in both EDTA and PAXgene systems, thus allowing maximum flexibility for providers to gain access to the test. The test detected two times the number of confirmed CRC cases as CEA. PAXgene system demonstrated similar sensitivity and specificity as the EDTA system with the added benefit of lower LoD and ease of specimen shipment.

REFERENCES

[1] A panel of genes methylated with high frequency in colorectal cancer. BMC 2014, 14:54
[2] Validation of a circulating Tumor-Derived DNA blood test for detection of methylated BCAT1 and IKZF1 DNA. JALM 2017, 220: 165-175