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

John Alsobrook, Snigdha Gaur, Emily Kinnaman, Laura Schnetzler, David Murray, Sherin Alex, Robert Boorstein

1Clinical Genomics Pathology Inc., Bridgewater, NJ, USA and 2Clinical Genomics Technologies Pty Ltd, North Ryde, NSW, Australia

INTRODUCTION

Colvera™ is a qualitative epigenetic assay that detects circulating tumor DNA by measuring the methylation within two genes: BCA1 (Branch chain amino acid transaminase 1) and IZKF1 (Ikaro 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 assay 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.

COLVERA TEST DESCRIPTION

Blood is collected either in 1) two EDTA tubes, processed to plasma within 7 hrs of collection & shipped frozen or in 2) two PAXgene Blood ccfDNA tubes (Qiagen; Cat. No 768115; 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 QIAxymphony SP using the QIAxymphony DSP Virus/Pathogen kit (Qiagen; Cat. No. 937055) from 4 mL EDTA plasma and QiAsymphony PAXgene Blood ccfDNA kit (Qiagen; Cat. No. 768536) from 4.8 mL PAXgene plasma. Extracted DNA is bisulfite converted using Epitect Fast 96 Bisulfite Conversion kit (Qiagen; Cat. No. 59720) on QIAcube HT. Bis-DNA is measured in triplicate by real time rtPCR on LightCycler 480 II using custom methylation specific primers & probes (IDT) and QuantiTect Multiplex PCR No ROX Mastermix (Qiagen; Cat. No. 204743). Colvera test is called positive when ACTB (β-actin) is positive and any replicate of BCA1 or IZKF1 has a positive Ct.

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.

Precision & analytical sensitivity: pooled plasma from self-declared normal donors spiked with methylated human gDNA (mDNA) at different conc. [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 Colvera Test described.

RESULTS

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

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

Interfering Substances:

• Both validation studies: cholesterol (5 mg/dL), EDTA (20 mg/dL), genomic DNA (100 ng/mL), glucose (10 mg/dL), hemoglobin (1 mg/dL), BCP (0.4% v/v), triglycerides (12 mg/dL) & uoric acid (0.24 mg/dL).

• PAXgene validation: albumin (40mg/mL), bilirubin (0.2 mg/dL).

• EDTA validation: EDTA (20mg/mL).

Following interfering with the assay:

• EDTA validation: albumin (40 mg/mL) & bilirubin (0.2 mg/mL); 90% concord 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.

Stability in PAXgene ccfDNA tubes: Amount of ACTB increased over time, likely due to WBC lysis, but that had no impact on Colvera results.

CONCLUSION

Colvera assay was successfully validated in a CLIA-licensed clinical lab (#31JD122075) as a LDT in both EDTA and PAXgene systems, thus allowing maximum flexibility for providers to get access to the test. The test detected twice 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 BCA1 and IZKF1 DNA. JALM 2017, 2:20:165-175

