METHOD VALIDATION OF A LABORATORY-DEVELOPED BLOOD TEST FOR COLORECTAL CANCER

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BACKGROUND
A novel, fully-automated DNA methylation test (ColoVantage Plasma; CVP) has been developed and clinically evaluated in blood samples obtained from a cohort of 2,105 people. CVP detects two methylated DNA biomarkers, BCAT1 (branched chain amino-acid transaminase 1) and IKZF1 (ikaros-family zinc finger transcription factor 1), as well as ACTB (QC control). CVP detected 85 (66%) of the 129 colonoscopy-identified cancers with 94% specificity.

AIM
To validate the CVP test with respect to its analytical performance characteristics as per NPAAC requirements.

MATERIALS and METHODS
384 in vitro samples were generated and scrambled into 8 batches of 48 samples, each batch containing:
(i) 11 pooled-plasma samples from healthy donors, spiked with methylated DNA plus potential interfering substances at up to 25-fold their upper plasma level (matrix effects);
(ii) 22 pooled-plasma samples spiked with different amounts of methylated DNA to assess: limit of detection (LoD); sensitivity; specificity; and intra- and inter-batch precision;
(iii) 4 process controls (PBS/BSA spiked with 4ng/mL genomic DNA plus (POSCONT) or minus (NEGCONT) methylated DNA (500pg/mL)); and
(iv) 11 additional NEGCONT samples, to detect any contamination throughout the process.

• The samples were extracted and assayed by different operators (blinded to sample type) over different days using different batches of reagents.
• Method ruggedness was tested by varying bisulphite-conversion and PCR temperatures by +/- 1°C or times by +/- 10%, and PCR oligonucleotide concentrations by +/- 20%.
• Results were compared using appropriate statistical analysis.

RESULTS

1. Excellent Assay Repeatability

Result 2: No Blood Matrix Effect

Matrix effects: ACTB

- Plasma was spiked with 500pg/mL methylated DNA, and further supplemented with one of 10 substances (X-axis) at between 2- and 25-fold their upper normal plasma concentration (The Merck Manual).
- No substance significantly affected BCAT1 or IKZF1 signal.
- The only observed effect was expected; additional DNA (genomic DNA, or RBCs containing some WBCs) caused earlier ACTB Cts.

Result 3: Robust Assay Precision

- Only one of the nine datasets (BCAT1 100pg/mL spike) showed significant variation (ANOVA) across different days/operators/reagent sets.
- This spike is at the linear limit of the assay.

Result 4: Reproducible Process Controls

- Both Process Controls showed good repeatability (low %CV, Table above).
- One of 136 NEGCONT samples was 1/3 replicates positive for methylated IKZF1 (lower panel). This could indicate low-level methylation in the spiked background genomic DNA, or low-level contamination.

Result 5: Assay LoD of 6 copies/mL

Sample positivity at different methylated DNA spike levels

- An LoD of 18pg fully-methylated DNA/mL plasma was determined.
- This is equivalent to approximately 6 copies (3 diploid cell genomes) of DNA per mL of plasma.

Result 6: Rugged Method Performance

(i) bisulphite-conversion thermal cycling

(ii) qPCR cycling conditions

(iii) qPCR oligo concentration

- All results remained within specifications for any parameter variation.

CONCLUSIONS

• No tested substance interfered with CVP assay performance, nor did variation in cycling time, temperature, or oligo concentration.
• No statistically-significant difference was seen in intra- and inter-batch precision, nor due to different operator, reagent batch, or date, within the linear range of the assay.
• The LoD (95% chance of a positive result) was 18pg/mL plasma (approx. 6 copies; 3 cells).
• The ColoVantage Plasma test was successfully validated according to NPAAC requirements.