Validation of increased sample stability using the AccuCyte®-CyteFinder® CTC Platform to improve logistics in global clinical trials

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BACKGROUND

Analysis of circulating tumor cells (CTCs) by multiparameter immunofluorescence (IF) microscopy allows non-invasive characterization of cancer cell biomarker expression in real time. This information can prove helpful in prognosis, treatment selection, and stratification of cancer patients; and the flexibility in global logistics and testing for CTC analysis within clinical trials is of paramount importance to trial sponsors. In this work, we validated increased blood stability for sample shipment from clinical site to processing centers and determined the stability of banked samples over multiple years. Given clinical trial timelines, it is essential to have consistent performance of sample preparation over multiple years, to this end, we validated improvements to the sample preparation consumables which enable large scale production to support large clinical trials. These 3 improvements to our platform provide increased flexibility and reliability for CTC testing.

Our integrated platform comprises the AccuCyte® Sample Preparation System (AccuCyte) and CyteFinder® Imaging Instrument (CyteFinder). AccuCyte is a density-based unbiased isolation method that transfers nucleated cells from whole blood to slides for the characterization of CTCs and other rare cells. RarePlex® Panel Kits are IF staining reagents used on automated slide staining instruments to label cells to differentiate CTCs from white blood cells (WBC). CyteFinder is a seven-channel automated fluorescent imaging system that rapidly scans microscope slides and applies machine learning algorithms to identify CTCs. Together, these technologies provide an end-to-end solution for CTC characterization. For analysis, blood is drawn into AccuCyte blood collection tubes (BCTs) containing a preservative which maintains cell properties prior to processing onto slides. Once slides are prepared, they can be stored at -20°C without significant biomarker degradation. This flexible workflow allows investigators to bank samples for batch analysis and to begin sample collection prior to validating the IF assay to be used.

METHODS

This study was designed to evaluate: (1) stability time between collection in the AccuCyte BCT and sample processing; (2) performance of an improved version of the AccuCyte prep kit with higher nucleated cell isolation capacity; and (3) storage time that AccuCyte prepared slides can be banked frozen prior to staining. The study was performed using model CTCs and cancer patient samples. Metrics to determine performance were CTC recovery and mean fluorescence intensity (MFI) of biomarker expression.

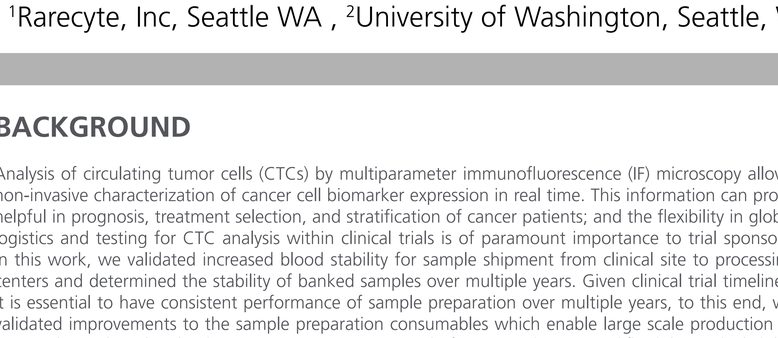

Figure 1. The RareCyte CTC assay workflow.

RESULTS

Our results demonstrate that the AccuCyte BCT preserves blood components for at least 5 days after collection without significant effect on CTC recovery or biomarker expression. The latest version of the AccuCyte prep kit demonstrated equivalent CTC recovery. Finally, accelerated-aging study results demonstrated that AccuCyte-prepared slides can be stored at -20°C for at least 4 years without significant effect on most biomarkers tested.

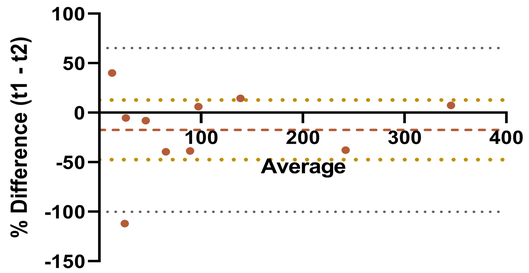

Figure 2. Comparison of CTC recovery in clinical samples. Percent difference in CTC recovery (t1 – t2) in breast and prostate clinical samples with >10 CTC/7.5 mL (n=10) between 24-72 hours post-collection and 120 hours post-collection. Dotted copper line indicates 24–72-hour to 120-hour bias (-17.3), gold dotted lines represent the 95% confidence interval (CI) limits of mean (-47.5, 12.8), gray dotted lines represent the 95% CI limits of agreement (-100.0, 65.3).

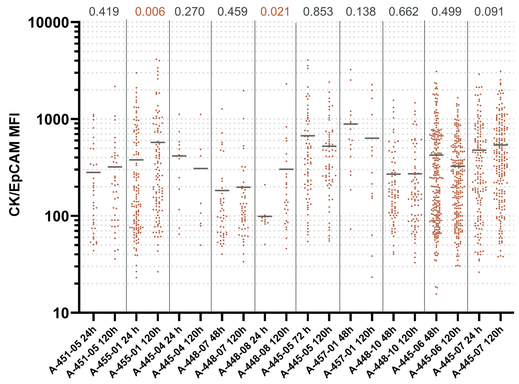

Figure 3. Epithelial biomarker stability in clinical samples. Single-cell CK/EpCAM MFI values of CTCs from 120-hour incubation testing on 10 patient samples. Individual horizontal lines represent mean value for that sample. p-value of a Mann-Whitney test to compare CK MFI between t=1 and t=2 is shown at top of each sample pair. Two sample pairs (copper) have p-value of <0.05. Of these, both are higher at t=2. ARv7, AR, ER and HER2 expression on CTCs and CD45 expression on WBCs was also tested and no significant difference was seen between the 2 timepoints (data not shown).

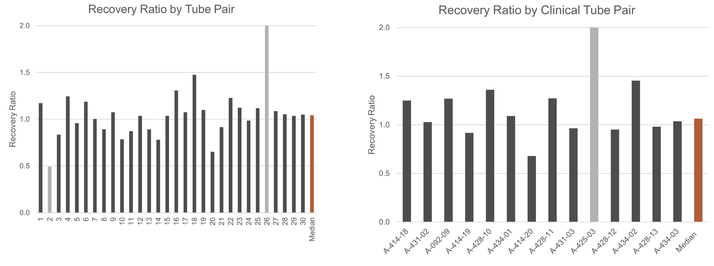

Figure 4. CTC recovery using improved AccuCyte kit. Left: mCTC Recovery Ratio (new kit/old kit) for 30 spike-in tube pairs. Median value for all pairs in orange. Outlier pair #2 (0.5) and #26 (23.9) in grey. Right: CTC Recovery Ratio (new kit/old kit) for 14 breast and prostate clinical tube pairs that had at least one partner with 10+ CTC per tube. Median value for all pairs in orange. Pair indicated in grey: 10 CTC with new kit and 3 CTC with old kit for ratio of 3.3.

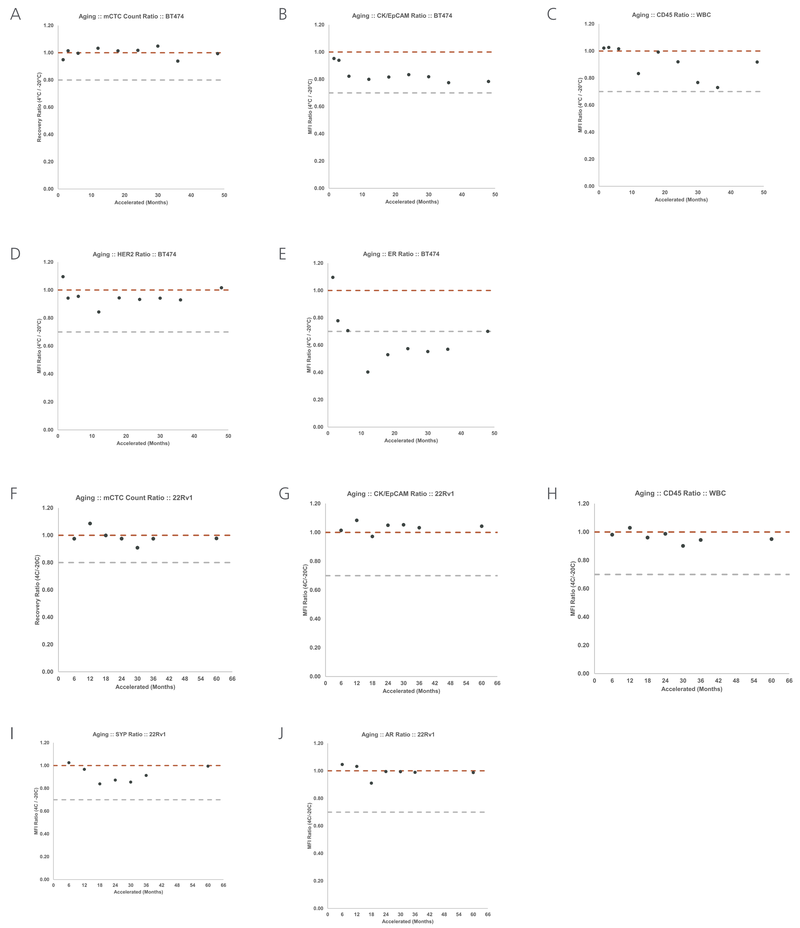

Figure 5. Frozen slide stability testing. Mean ratio (cell recovery or MFI) between slides aged at -20°C (real time) and accelerated aged at 4°C for either the equivalent of 4 years of storage for HER2/ER assay on BT474 cells (panels A-E) or for the equivalent of 5 years of storage for AR/SYP assay on 22Rv1 cells (panels F-J). Orange dotted line indicates no difference, gray dotted line indicates the lower limit of performance acceptability.

CONCLUSIONS

Enhancements to the AccuCyte-CyteFinder platform reported here greatly increase the flexibility for CTC analysis in global clinical trials by allowing longer periods of time before collected blood samples need to be processed, by extending the length of time processed slides can be banked before they are stained, and by implementing improvements to the sample preparation consumables.

ACKNOWLEDGEMENTS

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