High-recovery multiplex analysis of circulating tumor cells by density-based enrichment, automated platform immunofluorescence staining, and digital microscopy

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Abstract 3072

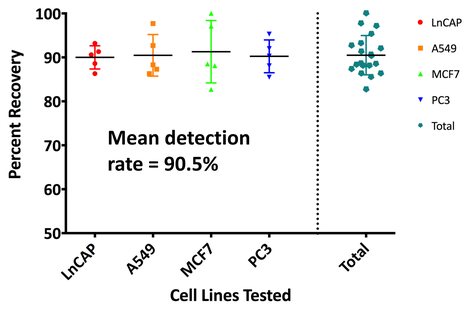
Background: Analysis of circulating tumor cells (CTC) is an intense area of diagnostic research that is rapidly evolving from prognostic to therapeutic applications. We applied three sequential technologies – density-based enrichment, automated immunofluorescence staining, and digital microscopy with image analysis – to the investigation of recovery rates from blood containing spiked-in tumor cells as a model of CTC.

Materials and Methods: Cultured MCF7, PC3, A549 and LNCaP cells were counted using VitroTube visualization. Precisely counted cells (mean: 116; range: 73 – 205) were spiked into normal blood samples in five replicates per cell line. Density-based enrichment was performed using the AccuCyte® tube, float and collector system in a single-tube, two-spin process. The buffy coat fraction was processed with an adherence solution and applied to charged microscope slides with a simple spreading device. After drying, slides were processed using the Ventana Discovery Ultra automated platform to apply fluorescently labeled antibodies to cytokeratin, CD45, and either EpCAM or EGFR, as well as Hoechst or DAPI nuclear dye. Slides were scanned on a CyteFinder® digital microscope and candidate CTCs were identified using CyteMapper® image analysis software. CTCs were verified by a reviewer based on morphology and expression of both epithelial and nuclear stains without CD45 expression.

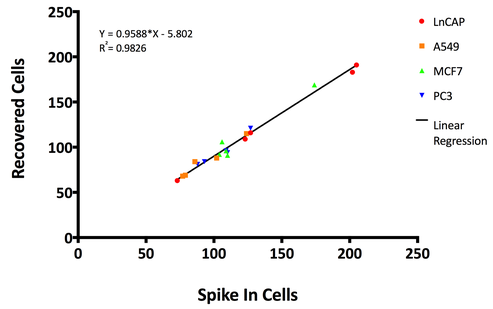
Study Design

- Precisely counted cells from cancer lines were spiked into whole blood
- Blood was processed using the RareCyte AccuCyte® system with automated immunofluorescence staining and digital microscopy analysis
- CTCs were identified and counted
- CTCs were assessed in triple-negative breast cancer clinical samples

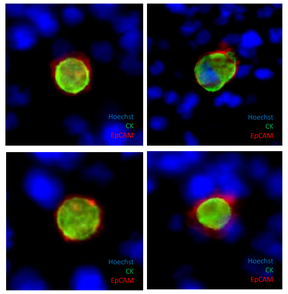
Spike-In Detection Rate



Spike-in/Recovery

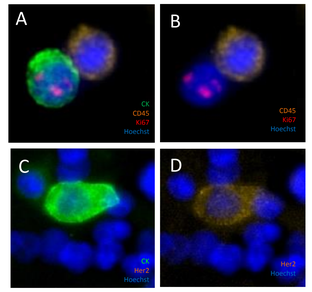


Application to Triple-negative Breast Cancer Clinical Samples

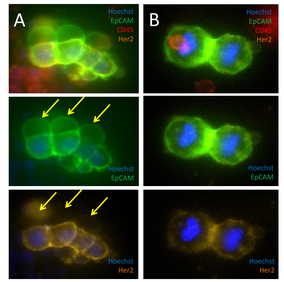


Representative CTCs from a triple negative breast cancer patient stained with antibodies to cytokeratin (green), EpCAM (red) and Hoechst (blue).

All images of clinical samples were obtained with a DeltaVision microscope (40X objective).



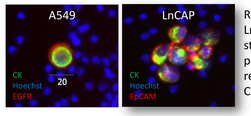
CTCs from a triple negative breast cancer patient stained with antibodies to EpCAM, cytokeratin, Her2, Ki-67 and CD45. A. CTC and WBC labeled with Ki-67 (red), cytokeratin (green), and CD45 (orange) antibodies, **B.** Same field as in (A) but shown without cytokeratin antibody staining. **C.** One CTC in a field of WBC showing cytokeratin antibody staining, **D.** Same field as in (C) showing Her2 antibody staining.



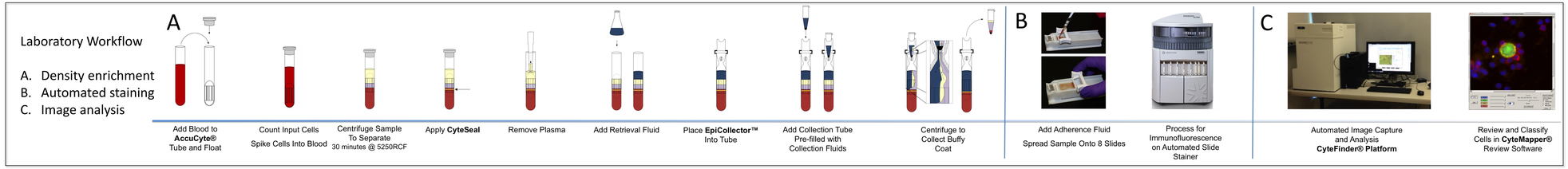
Clusters of CTCs from a triple negative breast cancer sample which were magnetically enriched and loaded on a wet mount. A. Cluster of 11 CTCs with homogeneous EpCAM expression but heterogeneous Her2 expression (arrows). **B.** Dividing CTCs with a WBC behind one of them.

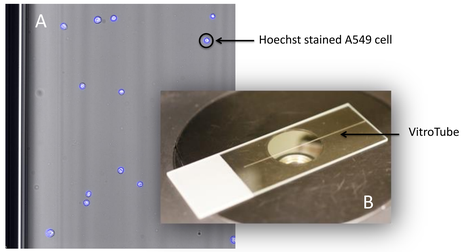
Results:

- Recovery of all spiked-in CTCs averaged 90.5% with SD = 4.5
- Mean recovery per cell line ranged from 90% to 91%
- Method was successfully applied to identification and biomarker analysis of CTCs from clinical triple-negative breast cancer samples

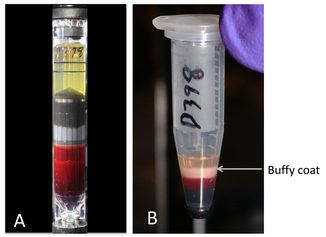


Representative A549 and LnCAP cells used in this study to determine the percentage of cells recovered using the CyteFinder® Platform



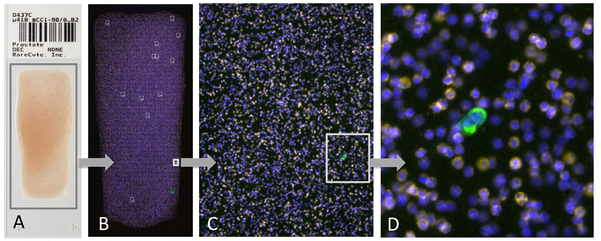


Live cells were freshly prepared as a suspension and the nuclei were fluorescently prelabeled before being drawn into a capillary tube (VitroTube). The VitroTube was then scanned and cells were counted on a fluorescent microscope. Cells were expelled into blood sample by flushing with PBS and the VitroTube was rescanned and counted to obtain the net count of the cells added to the blood. **A.** Fluorescent scan of Hoechst stained cells in VitroTube (transmitted light overlay). **B.** VitroTube on slide for scanning.



A. AccuCyte® tube and float after initial 30 minute centrifugation to separate 7.5mL blood sample into its component layers – plasma / buffy coat / red blood cells

B. Isolated buffy coat in collection tube after 5 minute retrieval centrifugation.



A. Buffy coat spread prepared for automated staining. In total, the buffy coat from 7.5mL of whole blood is spread across 8 slides. **B.** Scan comprised of 2419 individual 10x image panels. **C.** The single panel shows one such 10x image identified by the CyteMapper® software as containing a CTC. **D.** Cytokeratin positive CTC. Stains: DAPI (blue), CD45 (orange) and CK (green).

CyteMapper® review software displays objects of interest from whole-slide scans.

- Candidate CTCs are identified by the analysis software using criteria such as signal intensity, object size and cellular morphology.
- Images are presented for characterization and enumeration of CTCs.
- Each channel can be viewed independently or in any combination and objects can be shown in greater detail to resolve subcellular details.