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 ViTroveTube visualization. Precisely counted cells (mean: 118; 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 EGF, as well as Hoechst 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

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

Laboratory Workflow

A. Density enrichment
B. Automated staining
C. Image analysis

Spike-in Detection Rate

Cell Lines Tested

Precisely recovered

Spike-in/Recovery

Mean detection rate = 90.5%

Recovered Cells

Application to Triple-negative Breast Cancer Clinical Samples

C.

D.

E.

F.

G.

Buffy coat samples were processed using the AccuCyte® Platform immunofluorescence staining, and digital microscopy analysis. CTCs were enriched and loaded on a wet mount. A. Confocal imaging of CTCs stained with KRAS, HER2, CD45, and Hoechst dye. B. CyteFinder® image with cell nucleus, KRAS, HER2, and CD45 expression. C. Digital microscopy image of a CTC stained with KRAS, HER2, and CD45. D. Representative HER2+ CTCs from a triple-negative breast cancer patient stained with antibodies to cytoskeleton (green), HER2 (red), and Hoechst (blue). E. All images of clinical samples were obtained with a Deltavisor microscope (Oil immersion)

Clusters of CTCs from triple-negative breast cancer samples were stained for Ki67, CD45, and Hoechst, and imaged as a virtual mount. A. Cluster of 3 CTCs with luminal-like cytokeratin expression but heterogenous HER2 expression (arrows). B. Blending CTCs with a WELO behind one of them.

Live cells were heavily prepared in a suspension and the nuclei were fluorescently green with a green channel and were drawn into a capillary tube (ViTroveTube. This ViTroveTube was then scanned and cells were counted in a Fluoroscan microscope. Cells were expelled into blood sample using flushing with PBS and 1X ViTroveTube was mixed and counted to obtain the count of the cells added to the blood. A. Fluorescent scan of ViTroveTube Hassan cells mixed with a fluorescent dye (Hoechst). B. ViTroveTube side for scanning.