The RareCyte™ system for enumeration of circulating tumor cells that retains all nucleated cells for analyses and does not rely on capture of proteins expressed on cells

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Abstract
• Detection and molecular characterization of circulating tumor cells (CTCs) are useful for diagnosis, prognosis and measuring therapeutic response of malignant tumors.
• Many CTC detection methods are based on capture of cells that express EpCAM, limiting the method to cells with expression of this or other markers.
• The RareCyte™ system is based on density separation and the spread of nucleated cells onto an imaging surface.
• Our technology is not limited to a specific set of cancer biomarkers and requires no pre-enrichment step.

Technology Overview
The system uses a proprietary tube and float combination to separate the Buffy coat, which contains white blood cells and CTCs, from other blood components, and create a 30 µm layer for imaging. The entire Buffy coat is imaged in our automated fluorescence microscope.

Experimental Protocol:
1. Add antibodies to tube containing blood sample
2. Incubate for 1 – 2 hours
3. Insert float and then centrifuge for 30 minutes
4. Image on RareCyte™ scanner
5. Run detection algorithm to find CTCs
6. Review images to confirm identity of CTCs

Imaging Cancer Cells in Blood
Prototype Scanner

Spike-in Recovery of Cells

Spike-in results.
• MDA-MB-453 cells were spiked into 3 ml of blood from 14 healthy individuals and counted on the RareCyte™ system.
• Average recovery was 90%
• Sensitivity is one CTC within one billion blood cells.
• System could detect CTCs even 7 days after spiking in cells.

Open System

Multiple markers can be detected.
The RareCyte™ technology is an open platform that allows users to choose markers relevant to their interests. Nine markers have been used in our system to characterize CTCs: EpCAM (Panel A), PSMA (Panel B), Her2 (Panel C, in red), CD146 (Panel D, yellow), CEA (Panel E), CK (Panel F, in green), CD24, CD56 and EGFR.

Clinical Samples

Images of CTCs obtained with the RareCyte™ system.
CTCs have been identified in over 50 clinical samples from patients with prostate, breast, colorectal and skin cancer using the RareCyte™ technology. Panels A and B are clusters of CTCs from a breast cancer patient stained for CK in green and EpCAM in red. Panels C-I are CTCs from prostate cancer patients stained with CK in green, PSMA in yellow and EpCAM in red.

Conclusions
• The RareCyte™ technology is a new platform for CTC detection and characterization that does not rely on capture of cells through expression of a biomarker.
• Our system can image CTC clusters in the circulation. It is believed that these clusters have a higher metastatic potential than individual CTCs.
• We obtain a high recovery of spiked-in cells with virtually no false positives.
• Molecular characterization of CTCs can be customized and automated for deployment in clinical laboratories.