Investigation of custom biomarkers on circulating tumor cells from clinical samples using RarePlex® Developer Kits

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ABSTRACT

Enumeration and phenotypic profiling of circulating tumor cells (CTCs) can give important information about tumor progression, presence of therapeutic targets, and metastatic potential. New and informative cancer-specific biomarkers are being discovered at a rapid pace, so there is a strong need for tools that enable investigator driven assays to best study and utilize these biomarkers. RarePlex® Developer Kits enable the addition of user-selected antibodies against biomarkers of interest to a CTC detection assay. Here we demonstrate the application of RarePlex Developer Kits to study the presence of a variety of cancer related biomarkers. Using the Developer strategy, we present results for several biomarkers, including Synaptophysin (SYP), Vimentin, HER2, ER, PR, EGFR, Ki67, AR, ARv7, PDL1, and PSMA. We also characterized clinical samples from prostate (AR and ARv7) and breast (HER2 and ER) cancer patients. The biomarkers demonstrated proper localization on or within model CTC control cells when using default antigen retrieval and fixation conditions. For each biomarker, fluorescence intensity cut-offs that segregated negative and positive cell lines were statistically defined to maximize classification accuracy. For clinical samples, breast and prostate cancer sample staining showed expected localization based on available clinical information. In conclusion, RarePlex Developer Kits provide a flexible tool for custom CTC assay development that enables researchers to develop assays in their own lab for characterization of phenotypic heterogeneity.

METHODS

Blood samples spiked with positive and negative model circulating tumor cells (mCTC-positive and mCTC-negative) for each investigative biomarker were processed using the AccuCyte® Sample Preparation System. Slides were autostained with the RarePlex® CTC Panel Kit utilizing a three-channel CTC detection base: a nuclear dye, anti-CD45 antibody to exclude white blood cells, and cocktailed antibodies to cytokeratin (CK) and epithelial cell adhesion molecule (EpCAM). RarePlex Developer Kits were used to test biomarker expression of additional markers: HER2, ER, PR, Vimentin, SYP, EGFR, Ki67, AR, ARv7, and PSMA. For each biomarker, fluorescence intensity cut-offs that segregated negative and positive cell lines were statistically defined to maximize classification accuracy.

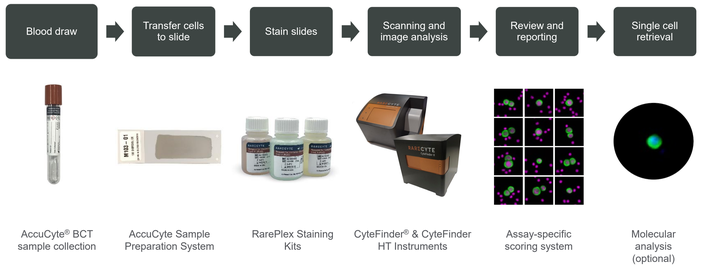


Figure 1: RareCyte liquid biopsy workflow. The RareCyte platform provides a blood-to-result CTC workflow with options to add custom biomarkers to enumeration Panel Kits and for single cell retrieval.

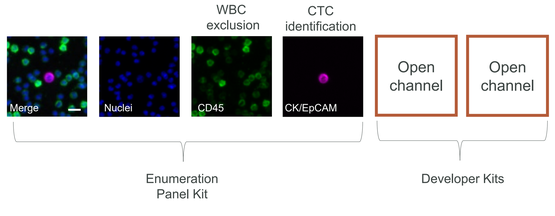


Figure 2: RarePlex Panel Kits are used with Developer Kits to create custom CTC assays. The Panel Kit is used to identify CTCs and Developer Kits are used to study up to two custom biomarkers of interest.

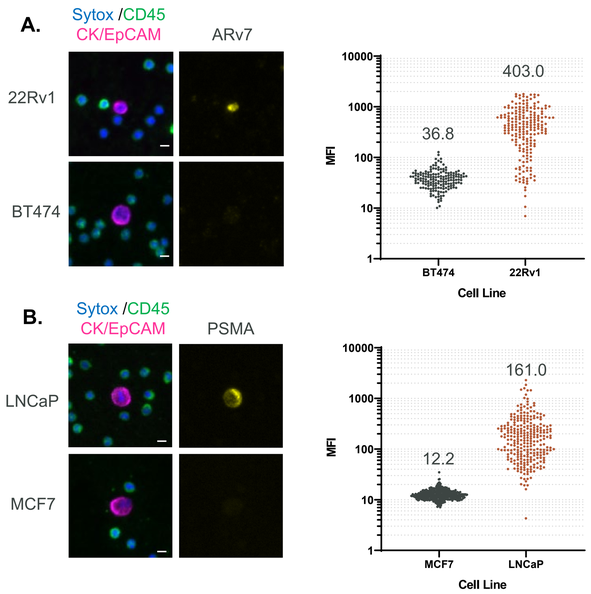
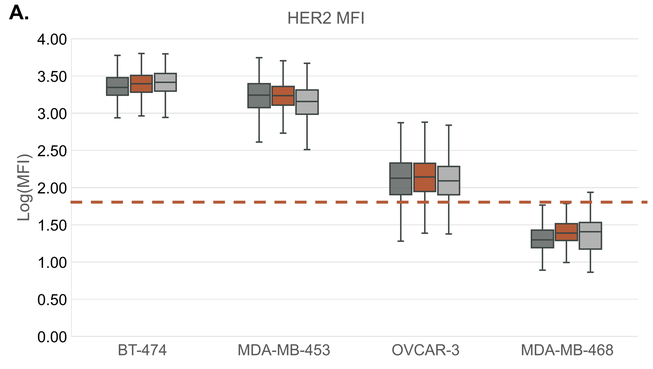


Figure 3: Clone specificity for biomarker-positive and -negative cell lines. Representative images of marker-positive (**A**: 22Rv1, **B**: LNCaP) and negative spike-in cells (**A**: BT474, **B**: MCF7) are shown on the left and dot plots of individual cell MFIs are shown on the right. Positive and negative cell lines showed the expected localization and expression, confirmed by fluorescence intensity values. Median cell intensity shown above the corresponding dot plot. Positive and negative images were scaled to the same display settings. Scale bars are 10 µm.



B.

% HER2 Positive			
BT474	MDA-MB-453	OVCAR3	MDA-MB-468
100.0	99.6	77.0	3.0

Figure 4: HER2 MFI distribution for mCTCs with a range of HER2 expression. (**A**) Four spike-in cell lines with known HER2 expression levels were compared: BT-474 (High), MDA-MB-453 (Medium), OVCAR-3 (Low) and MDA-MB-468 (Negative). Each cell line was spiked in to three separate healthy normal donors (indicated by bar color). MDA-MB-468 cells (marker-negative) were used to establish an MFI cut-off for marker positivity and applied to the other cell lines (dashed line) to determine the percentage of cells positive for HER2, as listed in the table (**B**). Whiskers are set to 1.5x Inter Quartile Range.

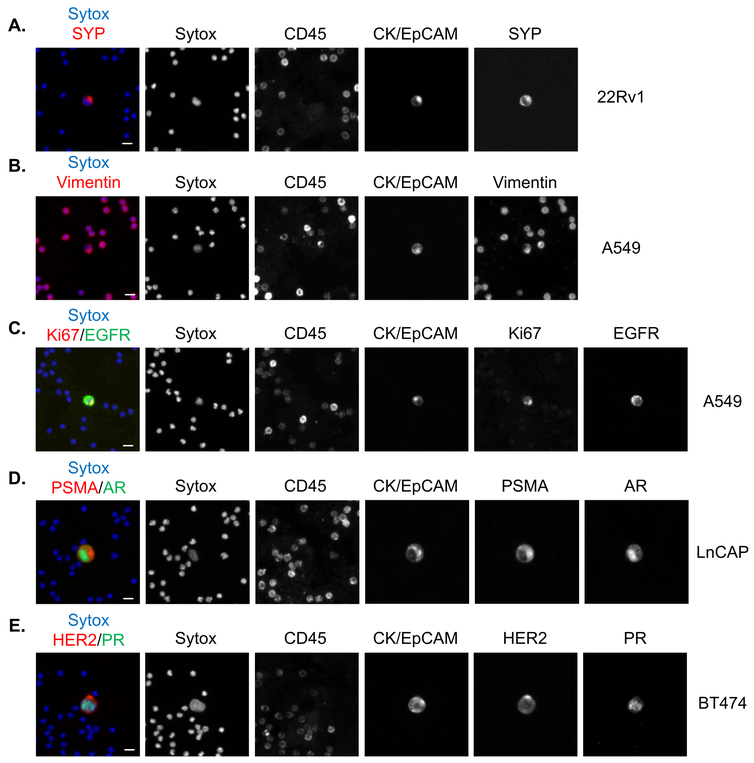
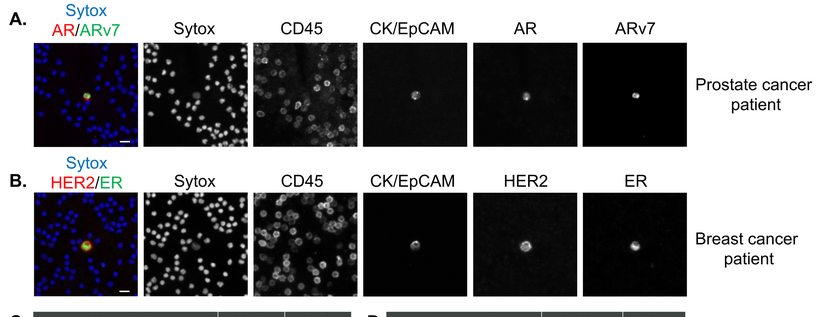


Figure 5: **mCTC biomarker expression revealed with Developer technology.** Individual channels and composite images of the indicated markers are shown. Scale bars are 20 µm.



C.

Prostate	AR+	AR-
ARv7+	4	0
ARv7-	26	13

D.

Breast	ER+	ER-
HER2+	13	3
HER2-	3	2

Figure 6: **CTC biomarker phenotypic diversity in clinical samples is revealed with Developer technology.** **A-B.** Individual channels and composite images of the indicated markers are shown. Scale bars are 20 µm. **C-D.** Tables with the number of CTCs identified with biomarker phenotypes, derived from prostate (**A**) and breast (**B**) cancer patients, respectively. Breast cancer patient had both ER+/HER2- and ER+/HER2+ tumors and this heterogeneity was also observed in the CTCs.

CONCLUSIONS

- RarePlex Developer Kits enable the addition of up to 2 custom biomarkers to a validated RarePlex CTC detection assay with minimal optimization.
- Developer Kits provide a flexible clinical tool for CTC assay development for non-invasive biomarker investigation