

Increased phenotypic depth for automated visual identification of biomarkers on circulating tumor cells by cocktailing epithelial markers EpCAM and cytokeratin

Yao Sun¹, Arturo Ramirez¹, Daniel Campton¹, Tanisha Mojica², Alisa Clein³, Celestia Higano³, Daniel Sabath³, Eric Kaldjian¹
¹RareCyte, Inc., Seattle, WA; ²Seattle Cancer Care Alliance, Seattle, WA; ³University of Washington, Seattle, WA

RARECYTE



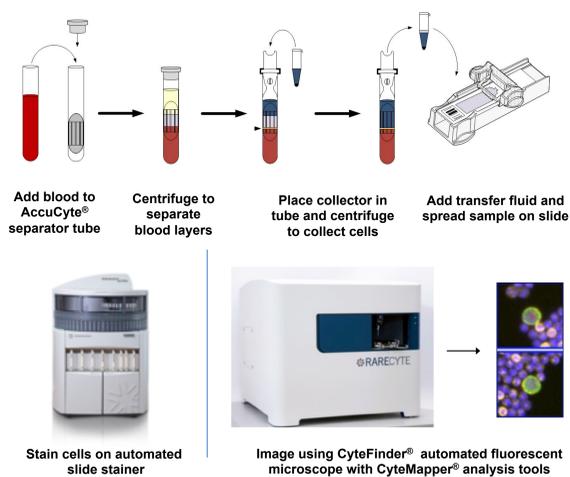
Background

Canonical methods for CTC identification have typically relied on co-expression of surface (EpCAM) and cytoplasmic (cytokeratin, CK) epithelial markers to separate them from white blood cells. RareCyte has developed AccuCyte® - CyteFinder® system, a platform for automated visual identification and retrieval of rare cells from blood by immunofluorescence (IF). RareCyte has also developed a 4-color standard panel for epithelial CTC detection (**CTC-4 panel**) in which EpCAM and CK are placed in individual fluorescence channels, allowing for identification of CTCs that express EpCAM and/or CK. Combined with recent expansion of CyteFinder imaging capabilities from 4 to 6 channels, this 4CTC panel can be combined with 2 additional markers for deeper phenotypic analysis. Given that our platform does not require co-expression of EpCAM and CK, RareCyte also developed a 3-color panel for CTC identification (**CTC-3 panel**) that combines detection reagents for EpCAM and CK detection reagents within a single channel, thus enabling 3 extra markers for deeper phenotyping. We tested feasibility of epithelial marker cocktailing with an assay to detect androgen receptor (AR) in prostate CTCs.

Methods

Normal human whole blood samples were spiked with prostate or breast cancer lines as model CTCs (mCTCs). Blood samples from University of Washington patients with advanced cancer were collected under an IRB-approved protocol. Blood was processed onto microscope slides using the AccuCyte sample processing system. Slides were then stained on an automated stainer using either the CTC-4 panel or CTC-3 panels combined with one, two or three additional biomarkers relevant to the mCTC. After development using spike-in models, the CTC-3 + AR assay was applied to a patient sample.

Sample preparation and analysis workflow



RareCyte CTC-4 assay					
Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6
Nucleus	CD45	CK	EpCAM	Biomarker 1	Biomarker 2

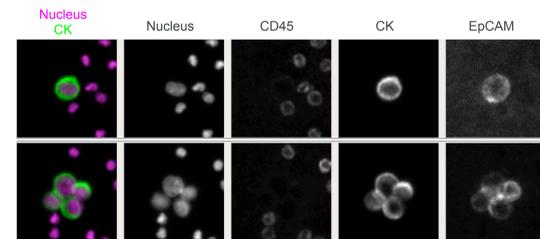


Figure 1. CTC-4 panel applied to spike-in samples. Table showing the CTC-4 panel (top) and individual channel images of SKBR3 mCTCs, processed using the AccuCyte system, and imaged using the CyteFinder system (bottom).

RareCyte CTC-3 assay with cocktailed epithelial markers					
Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6
Nucleus	CD45	CK/EpCAM	Biomarker 1	Biomarker 2	Biomarker 3

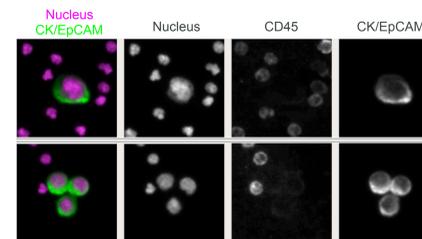


Figure 2. CTC-3 panel applied to spike-in samples. Table showing the CTC-3 panel (top) and individual channel images of SKBR3 mCTCs, processed using the AccuCyte system, and imaged using the CyteFinder system (bottom).

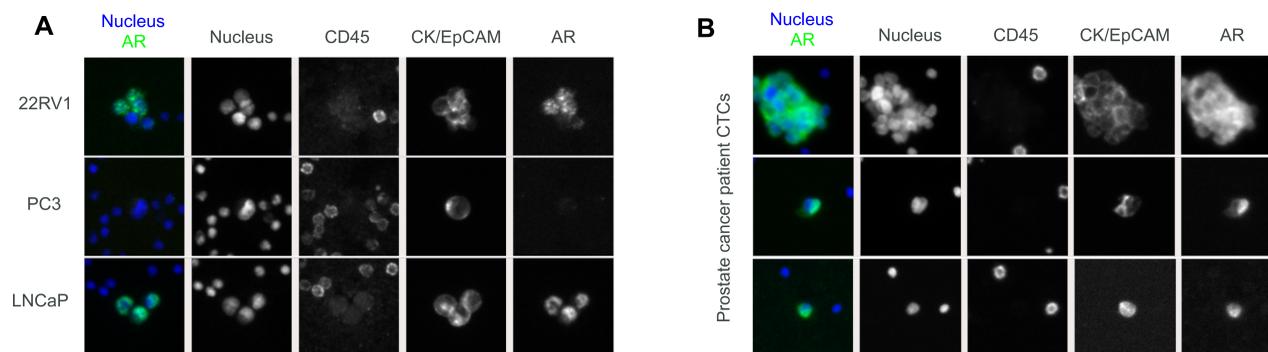


Figure 3. 4-parameter assay using epithelial cocktail: CTC-3 panel plus androgen receptor (AR) in additional biomarker channel. (A) CTC-3 + AR assay applied to spike-in samples. Note that PC3 does not express AR. (B) 3-plus AR assay applied to prostate cancer patient CTCs. 114 out of 160 CTCs identified were AR-positive. Note large AR-positive cluster in upper row.

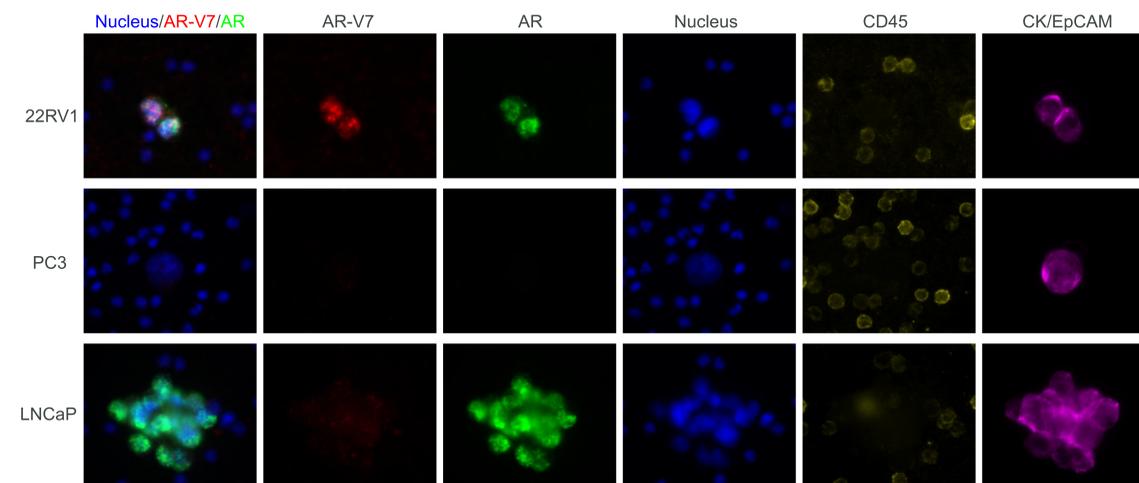


Figure 4. 5-parameter assay using epithelial cocktail: CTC-3 panel plus AR and splice variant AR-V7 in two biomarker channels. Note that 22RV1 expresses both AR and AR-V7. LNCaP expresses AR but not AR-V7. PC3 expresses neither AR nor AR-V7.

Breast cancer cell line phenotype		
Cell line	ER	Her2
BT474	+	+
MCF-7	+	--
SKBR3	--	+
MDA-MB-231	--	--

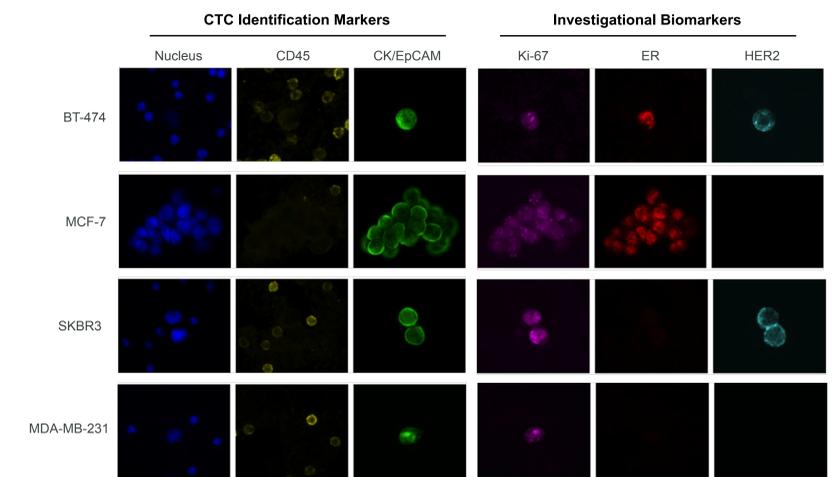


Figure 5. 6-parameter assay using epithelial cocktail: CTC-3 panel with estrogen receptor (ER) and Her2 receptor and Ki-67 in three biomarker channels applied to spike-in samples. Individual channel images of the indicated breast cancer mCTCs. The ER / Her2 expression patterns observed were consistent with reported phenotype (table above).

Results

No CTCs were identified in non-spiked normal donor blood samples (not shown). In spike-in models, the epithelial cocktail CTC-3 panel performed as well as the non-cocktail CTC-4 panel in identifying CTCs (not shown).

A 4-parameter **CTC-3 + AR** assay demonstrated AR expression in LNCaP and 22RV1 mCTCs but not in PC3 mCTCs, confirming specificity of AR staining. When the assay was applied to a blood sample from a prostate cancer patient with known high CTC count, 160 CTCs per mL were identified; of these, 114 were observed to be AR-positive (71%).

A 5-parameter **CTC-3 + AR / AR-V7** assay demonstrated AR-V7 expression in 22RV1 mCTCs but not in LNCaP or PC3 mCTCs, confirming specificity of AR-V7 staining.

A 6-parameter **CTC-3 + ER / Her2 / Ki-67** assay demonstrated ER / Her2 expression patterns consistent with reported phenotype in BT474, MCF-7, SKBR3 and MDA-MB-231 cells.

Conclusions

Combining the epithelial markers cytokeratin and EpCAM into a single wavelength cocktail is feasible for IF identification of CTCs, allowing up to three additional channel for phenotypic investigation. In a patient with advanced prostate cancer with high CTC count, there was high incidence (71%) of AR expression in identified CTCs.