BACKGROUND

There is considerable interest in investigating circulating tumor cells (CTCs) for biomarkers that provide information on drug target expression, response to therapy, and metastatic potential. Currently, options for investigating biomarkers on CTCs are limited due to challenges of developing multiplexed assays. The RareCyte platform combines CTC sample preparation, multiparameter fluorescence staining, imaging, and single cell retrieval. For custom biomarker exploration, RareCyte’s Developer Kit technology enables the simple addition of biomarkers of interest to a base epithelial CTC Panel Kit.

METHODS

Blood samples spiked with positive and negative model circulating tumor cells (mCTC-positive and mCTC-negative) for each investigative biomarker were processed using AccuCyte® Sample Preparation System. Slides were auto- stained with the RarePlex® CTC Panel Kit utilizing a three-channel CTC detection assay, a nuclear dye, and anti-CD45 antibody to exclude white blood cells, and cocktail-labeled 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, EGFR, Ki67, AR, ARv7, and PSMA under various optimized conditions (antibody concentration, incubation time and temperature). Stained slides were imaged with a CyteFinder® Instrument. CTCs were identified using machine-learning-based algorithms and confirmed by visual review in CytelkB® software. Biomarker analysis was performed by visual observation and mean fluorescence intensity measurements on confirmed CTCs.

RESULTS

RareCyte Developer Kits were successfully tested on a broad range of biomarkers on cell line control samples with default antigen retrieval and fixation conditions. For each biomarker, fluorescence intensity cut-offs that segregated mCTC-positive and mCTC-negative cell lines showed the expected localization and expression, confirmed by custom biomarker investigation of circulating tumor cells using RarePlex® Developer Kits.

CONCLUSIONS

• RareCyte Developer Kits can be easily combined with a base epithelial CTC Panel Kit to stain an additional one or two user-selected markers with minimal staining cross-reactivity.

• We have successfully applied the combined kits to 8 markers with default antigen retrieval conditions that have shown the expected sub-cellular localization.

• Model circulating tumor cells are used to establish fluorescence intensity cut-offs for each biomarker, and additional markers: HER2, ER, PR, EGFR, Ki67, AR, ARv7, and PSMA under various optimized conditions (antibody concentration, incubation time and temperature).

• For custom biomarker exploration, RareCyte’s Developer Kit technology enables the simple addition of biomarkers of interest to a base epithelial CTC Panel Kit.

• Custom assays created by combining Developer Kits and Panel Kits have been applied to clinical samples to characterize the expression of biomarkers of interest on CTCs.