Application Note

Accurate and precise spike-in method for CTC validation studies with cellenONE®

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Abstract

Analysis of circulating tumor cells (CTCs) from a routine blood draw has great potential for cancer diagnostic and treatment monitoring applications. The rarity of CTCs, often present at a frequency of 1 CTC per 10 billion red blood cells, demand technologies such as RareCyte's AccuCyte® - CyteFinder® system which combines exquisite sensitivity and specificity with a reproducible end-to-end workflow. RareCyte develops tools and assays for CTC enumeration and biomarker expression analysis for breast, prostate, and lung cancers. As diagnostics and clinical trials require accurate, precise, and reproducible CTC detection, rigorous assay validation is essential. For analytical validation of enumeration accuracy, it is critical to generate surrogate blood samples with known numbers of model CTCs (mCTCs) at levels near the limit of detection for the assay. These studies require an accurate and precise number of spike-in samples performed in a reproducible manner which is not possible by either serial dilution or flow cytometry approaches.

Developed by Cellenion, the cellenONE® instrument is a single-cell isolation and dispensing technology using automated image recognition to accurately ensure that each dispensed droplet contains only one cell. While the cellenONE is primarily used for single cell analyses and cell line development, Cellenion and RareCyte have collaborated to develop a new application of this technology. Here we demonstrate how RareCyte employed the cellenONE instrument to generate samples required for CTC assay validation. The cellenONE instrument proved essential to accuracy studies and is now the system of choice for mCTC sample preparation for these studies

Methods

A customized sample deck was designed for the cellenONE to enable cell deposition onto both microscope slides and into RareCyte's AccuCyte Blood Collection Tubes (BCTs) containing 7.5 ml of whole blood (Figure 1). In these studies, cellenONE was programmed to dispense 100 cells per slide or 5-8 cells per BCT. Blood samples were processed to slides using the AccuCyte system. mCTCs on all slides were counted after processing with RareCyte's CTC enumeration assay (Figure 1).

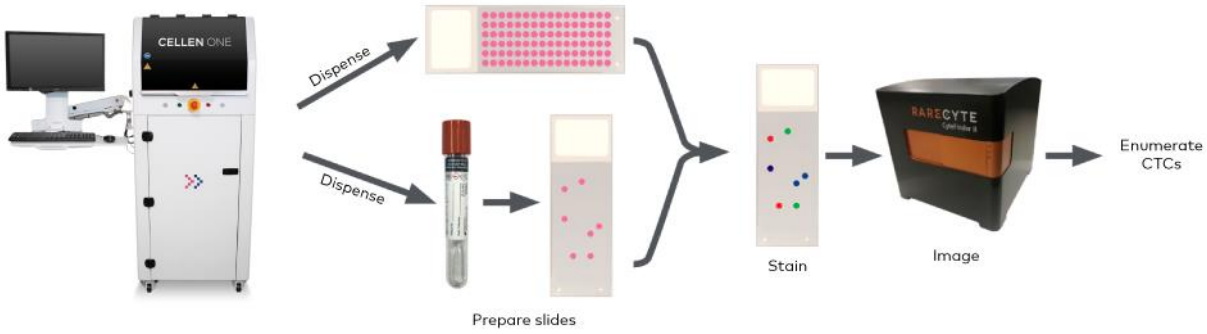

Figure 1 5-step CTC enumeration validation workflow. Dispense cells with cellenONE onto slides and into BCT; process BCT to slides with AccuCyte system; stain slides with RarePlex® Enumeration Panel Kit; image and analyze with CyteFinder Instrument to count CTCs.

Results and discussion

The cellenONE method allowed for dispensing known numbers of cells in a clinically significant range with a high degree of accuracy and precision, allowing standardized sample preparation techniques required for assay validation studies. The accuracy of the cellenONE system was verified by printing single cells directly to slides (Table 1). We then used the cellenONE to spike in limiting numbers of mCTCs into blood tubes. Processing these samples with the RareCyte CTC assay resulted in exquisite recovery rates (Table 2).

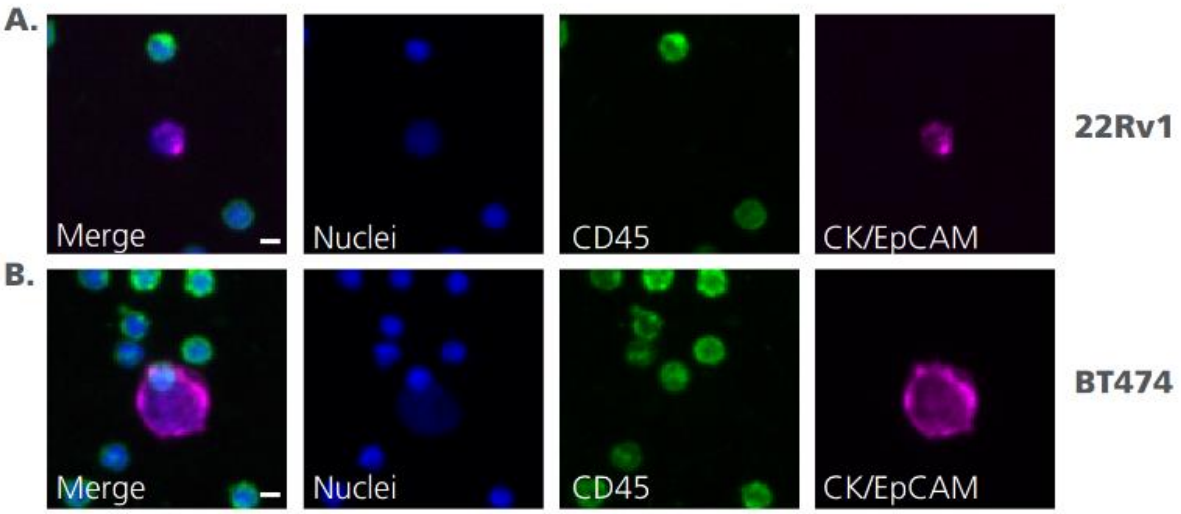

Figure 2 mCTC images from cellenONE spike-in assay. CTCs are identified by the presence of a nucleus, localization of Cytokeratin (CK) to the cytoplasm or Epithelial Cell Adhesion Molecule (EpCAM) to the cell membrane, and absence of CD45 localization. **A.** 22Rv1 mCTC spike-in assay. **B.** BT474 mCTC spike-in assay. Scale bar represents 5 µm.

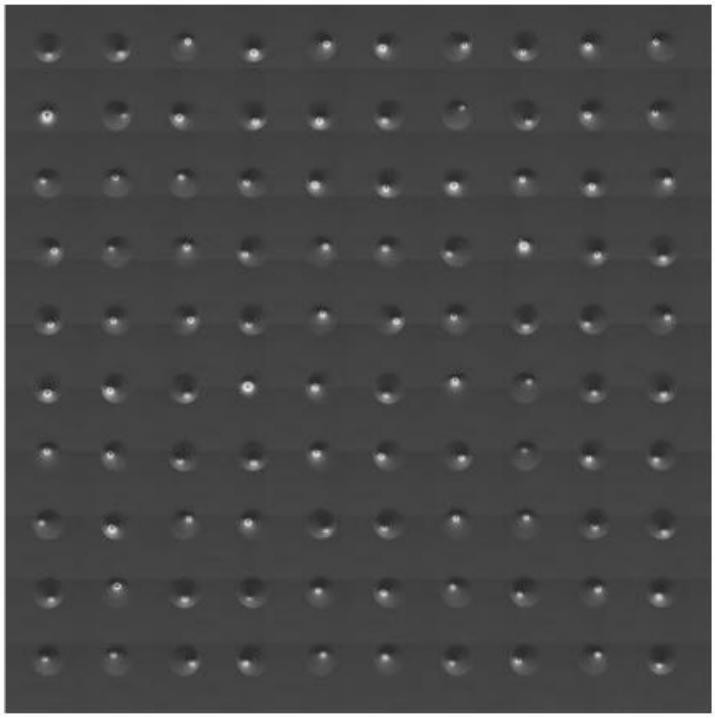

Figure 3 Image of 100 mCTCs printed to a slide.

Replicate	Print #	mCTC Count
1	100	100
2	100	100
3	100	100
4	100	100
5	100	99
6	100	99
7	100	100
8	100	99
Total	*800*	*797*
Recovery		*0.99*

Table 1 mCTC count for slides printed with 100 mCTCs.

Replicate	Spike-in #	mCTC Count
1	8	8
2	5	5
3	5	5
4	5	4
5	5	5
6	5	4
7	5	5
8	5	5
9	6	6
Total	*49*	*47*
Recovery		*0.96*

Table 2 mCTC count from deposition of 5-8 cells per BCTmCTCs.

Conclusion

Because clinical diagnostics demand accuracy, precision, and reproducibility, RareCyte performs rigorous validation to ensure it delivers high-performance CTC assays. For accuracy validation studies, RareCyte required an accurate and reproducible system for dispensing single mCTCs. As neither flow cytometry nor serial dilution assays are suitable for single-digit cell deposition at high accuracy, RareCyte utilized cellenONE technology for assay validation studies. cellenONE is now an integral component of RareCyte accuracy studies that are performed on every commercially developed CTC assay.

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