An integrated workflow for the isolation, automated detection & enumeration of Circulating Tumor Cells

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1. INTRODUCTION

Circulating Tumor Cells (CTCs) offer great potential to transform the standard of care for cancer patients. Enumeration and characterization of CTCs can be used to assess patients' response to treatment and inform clinical decisions during disease progression [1,2].

While the number of CTCs is associated with a patient's survival prognosis, the scoring of CTCs can be prone to inter-reader differences [3]. The integration of CTC isolation with an automated, high-resolution imaging system with advanced analytical capabilities is necessary to achieve objective scoring.

Here we describe the development of a sample-to-result workflow from CTC isolation to enumeration and characterization by combining Vortex's automated CTC isolation system [4] with the Axon Dx's nCyteDx® scanning platform [5,6].

2. METHODS

For workflow optimization and validation, peripheral blood from healthy donors was drawn in ACD-A or CEE-Sure tubes, and 4 mL aliquots were spiked with 50 cancer cells from different cancer types, including breast (MCF7, SK-BR-3), lung (HCC827), and prostate (PC-3).

CTCs were isolated from whole-blood using Vortex's VTX-1 (High-Recovery mode), immobilized on a microscope slide, or released on well-strips (Control), fixed and stored at -20°C (slides) or at 4°C (well-strips) until analysis.

The prepared slides were thawed, rehydrated, and labeled with a cocktail of conjugated antibodies directed against cytokeratins and WBC markers (nCyte nPAC™), counterstained with a nuclear dye (DAPI), and analyzed on the nCyte Dx® automated scanning fluorescent microscope.

The nCyte Dx® platform rapidly scanned and identified the cells with cancerous characteristics and recorded their positions on the slide. Then the imaging and data collection nAble® software, using AI-based algorithms for detection of CTCs, provided cellular analytics and high-resolution images (40X) of intact CTCs., i.e., nucleated cells, negative for WBC markers, and positive for CK.

The Cell Recovery (i.e. the #cells enumerated/#cells spiked in) calculated from the automated enumeration was compared to the Cell Recovery of matching controls, stained, and enumerated with a reference protocol [7] ("operator-based enumeration").

3. WORKFLOW

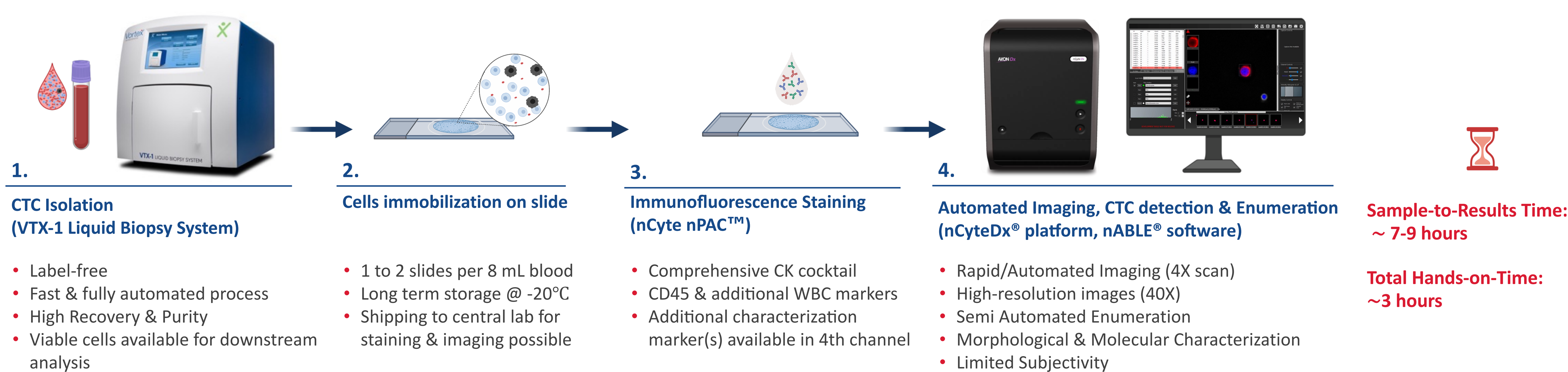

Figure 1: Schematic representation of the integrated workflow for the isolation of Circulating Tumor Cells and their automated imaging, detection and enumeration.

4. RESULTS

1 Workflow optimization and validation

- Full integration of the two platforms was achieved by optimizing the imaging/detection strategy to accommodate the high purity of the CTC sample collected on the slide.
- In controlled experiments, no significant difference in MCF7 cell recovery (an indication of cell loss) was observed between the automated and operator-based enumeration of matched samples (unpaired t-test, two-tailed t(22)=0.8874, p=0.3844). (Figure 2A).
- The nABLE® software algorithm detected "CTCs" with high accuracy for all cell lines tested (Figure 2B). No significant difference were reported between cell recoveries derived from automated and operator-based counts.
- Both cells with high (SK-BR-3; MCF7) and low (PC-3) CK expression were successfully detected by the algorithm.

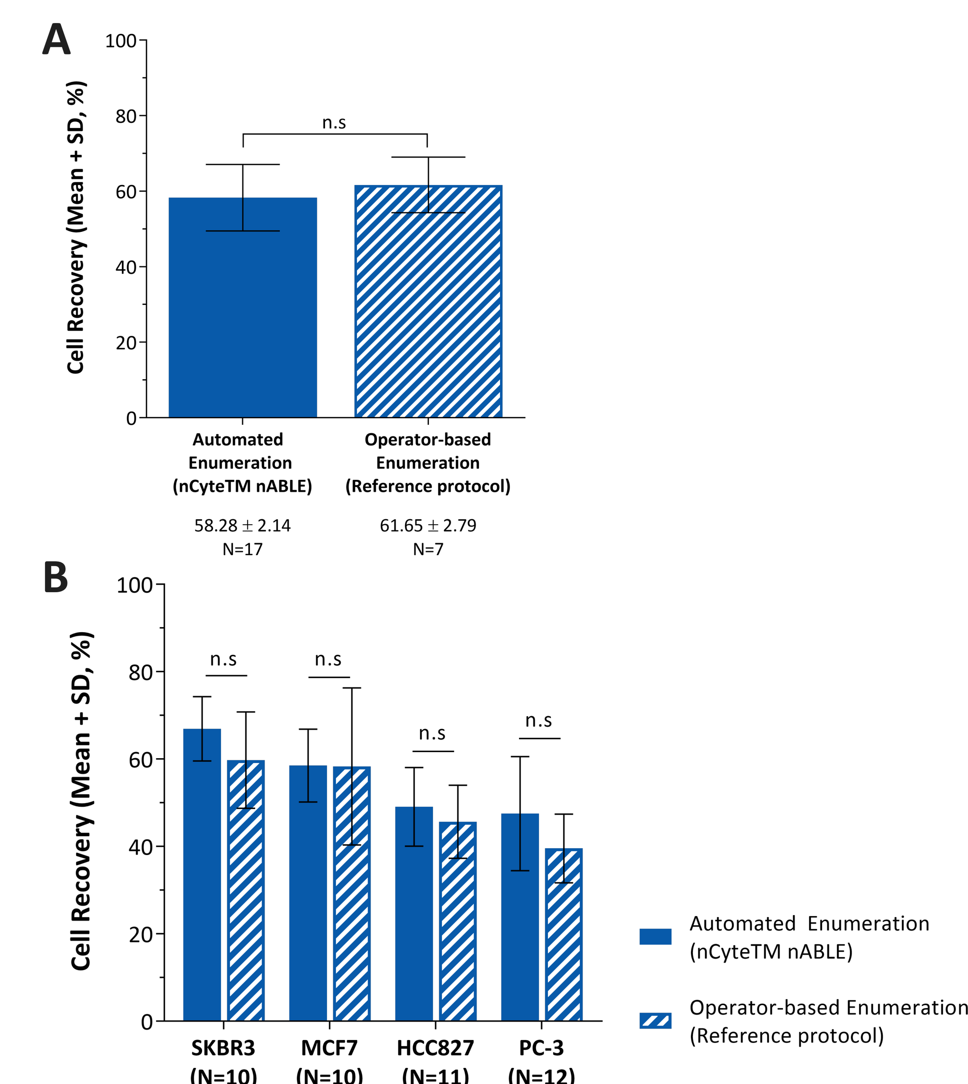

Figure 2: Comparison of Cell Recoveries calculated from automated and operator-based enumeration (matched controls) data.

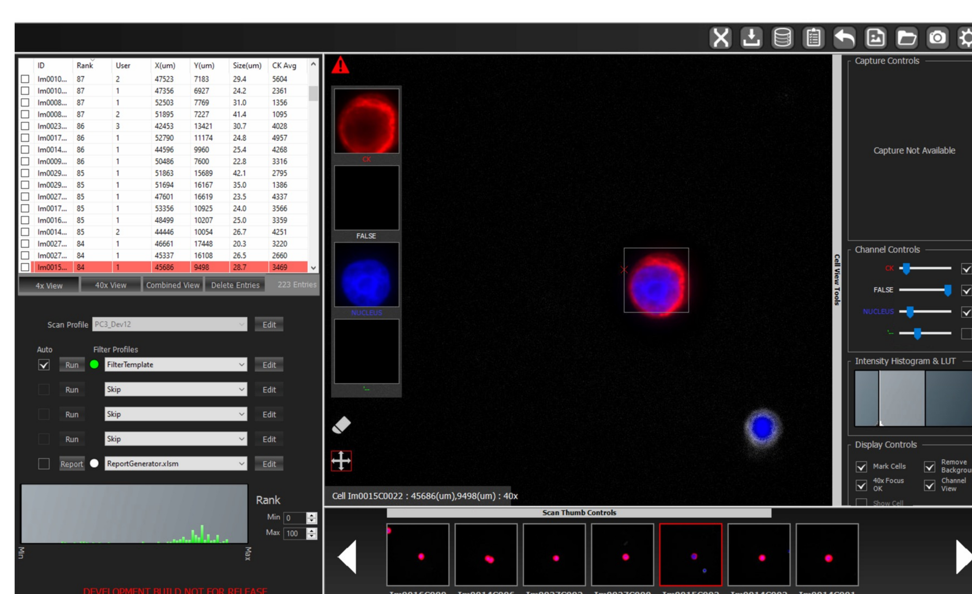

Figure 3: Example of a cell identified as "CTC" as seen in the nABLE® software user interface.

- CTCs detected during automated scan are presented on screen for operator review, confirmation and report.

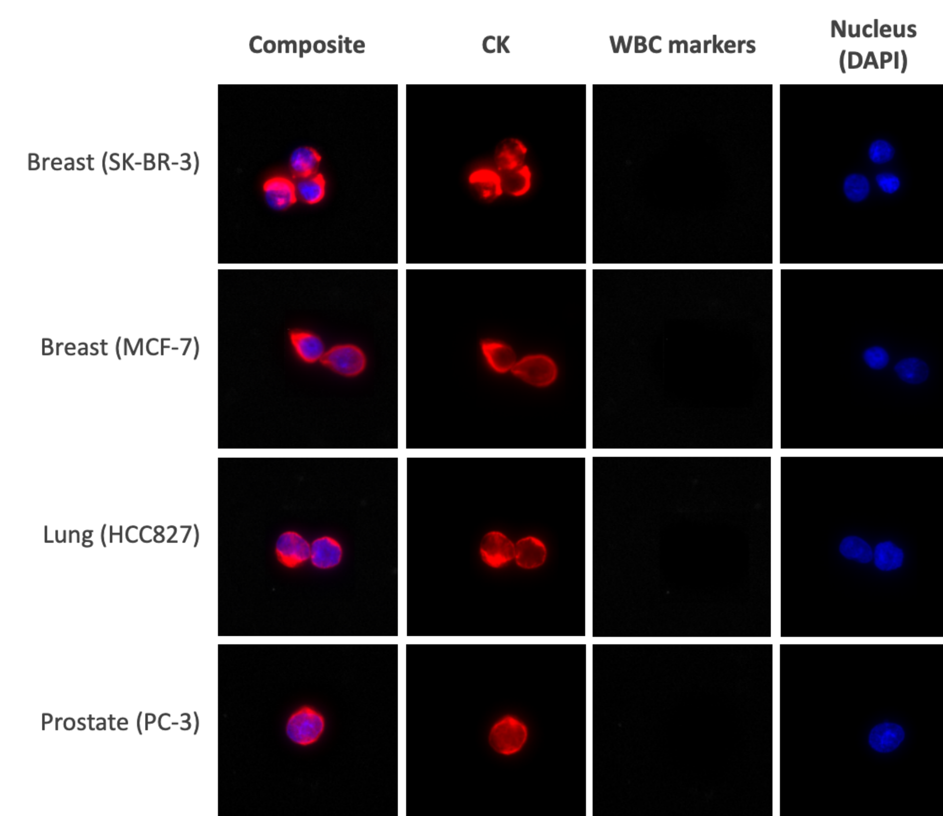

Figure 4: Representative high-resolution images (40X) of various cancer cells, isolated from whole-blood with the VTX-1 and detected/enumerated with AxonDx's nCyteDx® scanning platform.

2 Workflow performance are stable for up to 96 hours post phlebotomy

- Blood from healthy donors was drawn on CEE-Sure tubes and 4 mL aliquots spiked with 50 MCF7 cells on the day of the draw. CTC were isolated and enumerated from spiked samples at 24, 48, 72, and 96 hours using the workflow described herein.

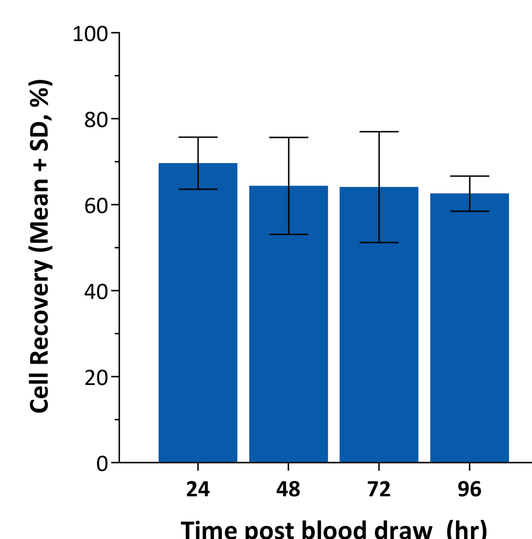

Figure 5: Comparison of Cell Recoveries of MCF7 cells over time. N=3 for each timepoints.

- No significant difference in Cell Recovery (%) were observed between the different timepoints (one-way ANOVA, F(3,8)=0.3292 , p=0.8046).
- The average cell recovery (in %) across all timepoints was 65.2±8.4 (vs 65.3±5.8 for matched controls manually enumerated; data not shown).
- ➢ Workflow performance was stable for up to 96 hours post phlebotomy.
- ➢ Patients samples can be shipped to a central lab for isolation, enumeration and characterization.

6. CONCLUSIONS & FUTURE DIRECTIONS

- We have achieved seamless integration of the VTX-1 Liquid Biopsy System with Axon Dx nCyteDx® automated downstream analytics.
- The high purity of the CTC sample (<2,000 WBCs per 8 mL blood analyzed) combined with rapid automated imaging and semi-automated enumeration significantly shortened analysis time with imaging and enumeration of CTC isolated from 1 tube of blood typically completed in 1 to 2 hours.
- Workflow performance was stable for up to 96 hours post phlebotomy which is critical for clinical trials performed across multiple clinical sites.
- The availability of standardized, unbiased, and fully validated methodologies for CTC isolation, detection & enumeration such as the integrated workflow presented here is needed for objective scoring.
- Additional studies are ongoing to optimize and validate the proposed workflow with clinical samples.

7. REFERENCES

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