Parsortix™ system: Analytic performance evaluation using ovarian cancer cell lines

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INTRODUCTION

Ovarian cancer has the worst prognosis among gynecologic malignancies, in part due to presentation with later stage disease in a majority of women. Much effort has been expended towards early detection, but it remains a significant issue and challenge. One approach which may have potential in improving the detection of ovarian cancer, particularly in women who present with an adnexal or pelvic mass, is the isolation and interrogation of circulating tumor cells (CTCs) from peripheral blood. The microfluidic Parsortix™ system isolates rare cells from biological fluids, particularly blood, on the basis of cell size and deformability.

The primary aim of the present study was to evaluate the performance of a modified Parsortix system for the isolation of ovarian cancer cell lines spiked into blood drawn from healthy volunteers (HVs). Parameters such as sensitivity, determined by dilution linearity, reproducibility and stability (time after blood draw) were assessed. In addition, the ability to molecularly interrogate Parsortix harvests by HyCEAD™ Ziplex® and RT-qPCR for the CTC-specific genes **E**pithelial **C**ell **A**dhesion **M**olecule (EpCAM) and **C**yto**K**eratin (CK) was assessed.

Figure 1. Modified Parsortix Instrument
Parsortix instrument with a number of external modifications highlighted. Certain internal changes were also made in order to improve speed and reliability.

METHODS

- 7.5-10mL whole blood samples were collected from female healthy, normal volunteers in separate K_2EDTA vacutainers.
- Samples were spiked with approximately 5-100 CaOV3 or SKOV3 cells (dependent on experiment). Cells were either pre-labelled with CellTracker™ Green CMFDA dye, or left unlabelled (dependent on experiment).
- Spiked blood samples were processed with the Parsortix system (Figure 1). 1 mL of 1x DPBS was added to all blood samples directly before processing.
- Enriched cells (Parsortix harvest) were either counted to assess cell recovery, or collected into lysis buffer and processed using either ANGLE's HyCEAD Ziplex (Hybrid Capture Enrichment Amplification and Detection) assay or using an in-house developed RT-qPCR assay (Figure 2).
- For Parsortix harvest counting experiments, CellTracker™ Green-labelled cells (CaOV3 or SKOV3) were used, and counted using fluorescence microscopy. Average blood separation times and numbers of nucleated cells in the harvest were also assessed.
- The HyCEAD Ziplex assay is a multiplex PCR assay allowing for the interrogation of 100 or more mRNA species.
- The in-house developed RT-qPCR assay allows for the assessment of up to 9 genes. The assay consists of the following steps: mRNA extraction, cDNA synthesis, pre-amplification, and PCR. The assay uses TaqMan™ primers/probes, which can be chosen based on the requirements of the user.

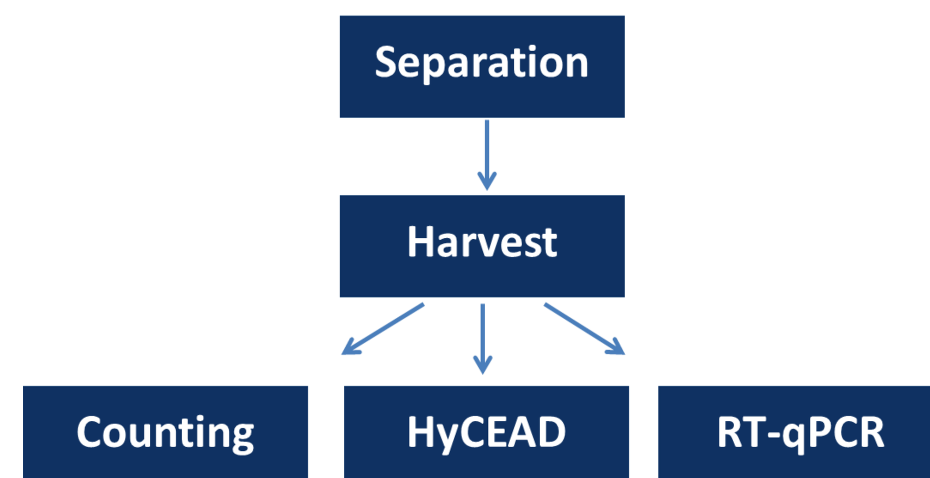

Figure 2. Experimental Schema

RESULTS

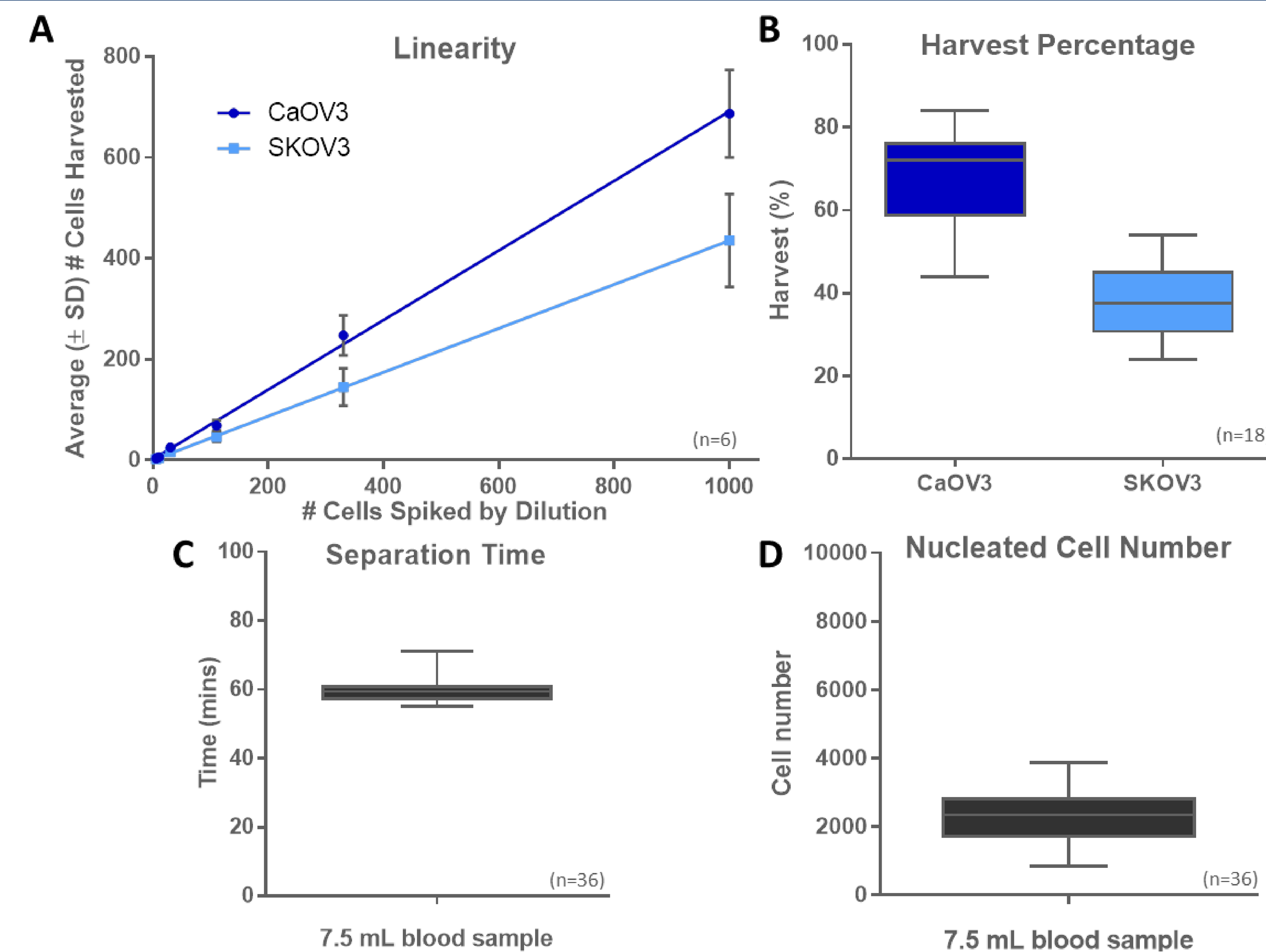

Figure 3. Cell Line Linearity and Recovery
(A) ~1000, 330, 110, 30, 10 or 5 CaOV3 or SKOV3 cells were spiked into 7.5 mL EDTA blood samples (0-8h). Cell recovery was linear for both cell lines. Error bars represent standard deviation. **(B)** ~100 CaOV3 or SKOV3 cells were spiked into 7.5 mL EDTA blood samples (0-8h). The mean recovery of CaOV3 cells was ~68%, while the mean recovery of SKOV3 cells was ~38%. **(C)** The mean separation time for 7.5 mL, 0-8h EDTA blood samples was ~60 min. The total sample processing time was ~92 min (includes cassette priming and instrument cleaning). **(D)** The average number of nucleated cells in the Parsortix harvest was ~2500 cells (7.5 mL, 0-8h EDTA blood samples). Error bars represent min and max (B-D).

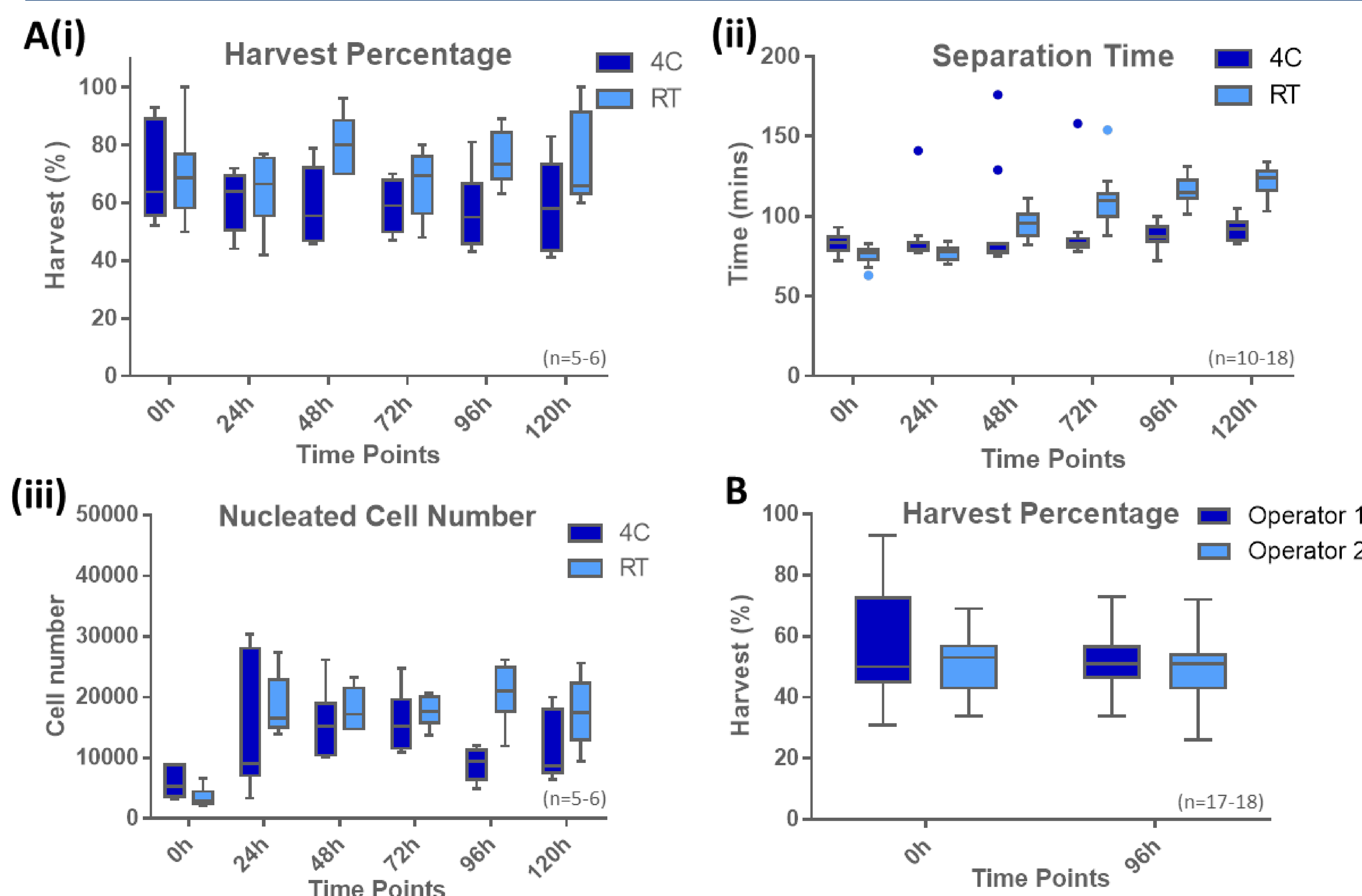

Figure 4. Blood Stability and Reproducibility
(A) ~100 CaOV3 cells were spiked into 10 mL EDTA blood samples at time 0h. Spiked blood samples were stored at either room temperature (RT) or 4C for up to 120h, and then processed using Parsortix. (i) The mean recovery of the CaOV3 cells at each time point was consistent for each of the storage temperatures. (ii) The mean separation time for blood stored at RT increased over time from ~76 min (0h) to ~120 min (120h). The mean separation time for blood stored at 4C was consistent across the time points at ~90 min. (iii) Nucleated blood cell numbers increased as a result of blood storage. **(B)** ~60 CaOV3 cells were spiked into 10 mL EDTA blood samples at time 0h, stored at RT and processed at either 0h or 96h. The experiment was conducted by two independent operators. Similar recovery of CaOV3 cells was achieved by both operators at both time points. Error bars represent min and max (A-B).

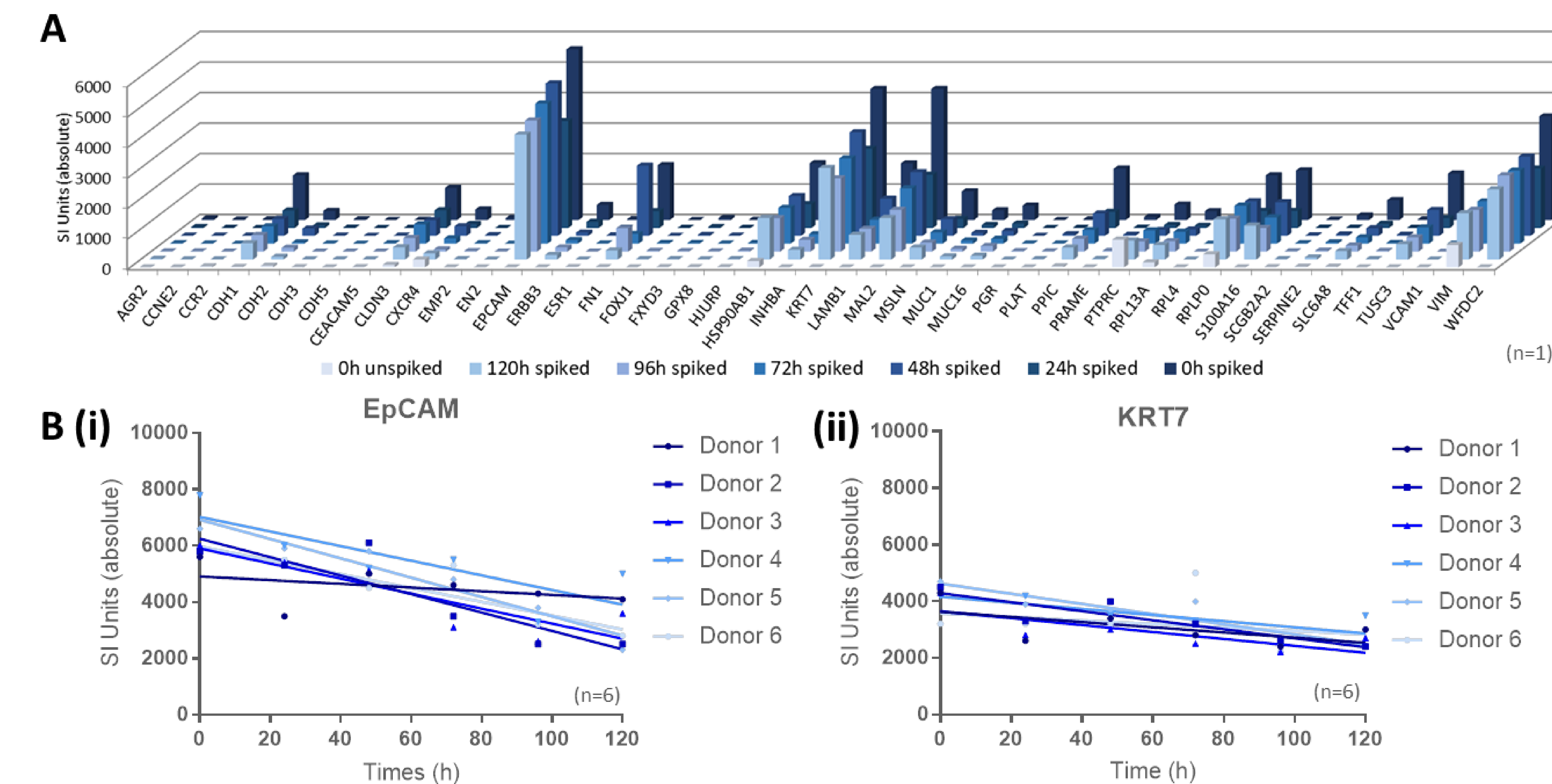

Figure 5. Parsortix + HyCEAD Ziplex
~60 CaOV3 cells were spiked into 10 mL EDTA blood samples at time 0h, and stored at RT for up to 120h. Blood samples were processed using Parsortix, and the harvested cells were lysed and assessed using HyCEAD Ziplex. **(A)** Gene expression data from a subset (45) of the assayed genes, demonstrating that expression of select genes can be observed at all time points and the pattern of expression is ostensibly constant. **(B)** By way of example, EpCAM and KRT7 expression levels decreased linearly as a result of blood storage but remained readily detectable up to 120h with RT storage; data presented for six individual HNV donors.

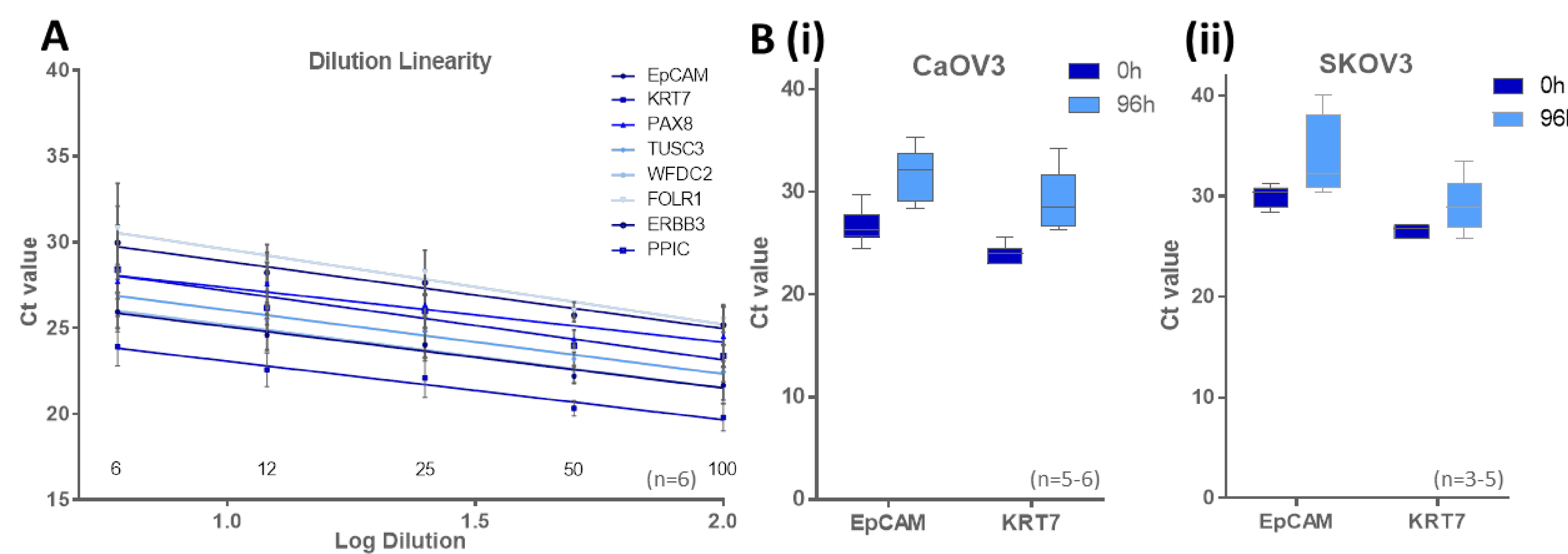

Figure 6. Parsortix + RT-qPCR
(A) ~100, 50, 25, 12, or 6 CaOV3 cells were spiked into 7.5 mL EDTA blood samples (0-8h), and processed using Parsortix. The harvested cells were lysed and assessed using RT-qPCR. A linear trend was observed for all genes assessed (EpCAM, KRT7, PAX8, TUSC3, WFDC2, FOLR1, ERBB3, PPIC). Error bars represent standard deviation. **(B)** ~10 CaOV3 or SKOV3 cells were spiked into 7.5 mL EDTA blood samples, stored at RT, and processed at 0h or 96h. The harvested cells were lysed and assessed using RT-qPCR. By way of example, EpCAM and KRT7 expression was observed at both time points, although Ct values did increase as a result of blood storage. Error bars represent min and max.

CONCLUSIONS

- EDTA blood samples could be stored for up to 120h and Parsortix-isolated cells could be assessed by counting, HyCEAD Ziplex, or RT-qPCR.
- Linearity was demonstrated through harvested cell counting and RT-qPCR when using the Parsortix system for isolation of CTCs.
- These data demonstrate both the robustness and sensitivity of the Parsortix system for the isolation and interrogation of CTCs.
- Further studies, specifically in the area of ovarian cancer, are ongoing.

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