Detection of EpCAM negative circulating tumor cells in CellSearch waste

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Introduction: Circulating tumor cells (CTC) measured with the CellSearch system in patients with metastatic carcinomas are associated with poor survival. The CellSearch system uses immunomagnetic enrichment targeting the EpCAM antigen. A frequently raised question is what the frequency and significance is of CTC that do not express EpCAM within the CTC population of the individual patient and between different patients. To investigate this, a device was constructed that collects the blood discarded by the CellSearch system and passes the blood through micro sieves with 5um pores to enrich for the larger CTC.

Methods: A sample collection device was attached to the waste line of the CellTracks Autoprep (AP). The blood discarded after immunomagnetic separation was detected in the waste line and collected into a separate 50ml conical tube for each patient sample. After collection, the blood was filtered through microfabricated silicon nitride filters with pore diameters of 5 micrometer (Vycap, Deventer, The Netherlands, www.vycap.com) with a pressure below 40mbar. To evaluate recovery on the filter the COLO320 (median size 11µm), SKBR3(16µm) and T24(16µm) cell lines where used. To evaluate recovery from the AP waste the SKBR3(57k EpCAM antigens) and T24(7k EpCAM antigens) cell lines where used. 300 cells where spiked in 7.5ml of blood collected in CellSave tubes from healthy volunteers. All samples were processed the day after collection.

Cell line selection

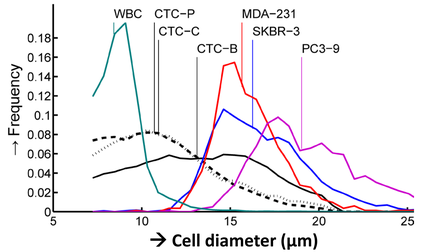

Figure 1: Cell size obtained by image analysis. The distributions are normalized to the area under the curve. Vertical lines indicate the median size for each cell type. CTC-B: breast CTC (N=34233) CTC-C: colorectal CTC (N=9734) CTC-P: prostate CTC (N=29358) [1].

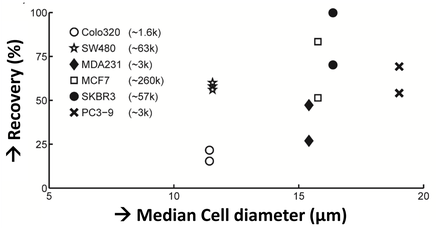

Figure 2: Recovery of cell lines on filters with 26.000 5um pores. The EpCAM antigen expression is noted in brackets behind each cell line. Adapted from [1].

To identify cell lines suitable for system characterization the size of CTCs found with CellSearch was compared with the size of cell lines. Size has some correlation with recovery [1]. This recovery together with EpCAM expression are important factors.

Sample collection

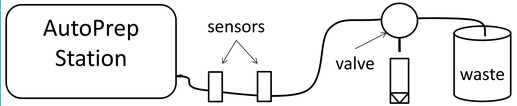

Figure 3: Schematic overview of the collection of discarded blood from the CellSearch System. Sensors detect the approach of a sample and valve is operated to transfer it to a 50ml conical tube.

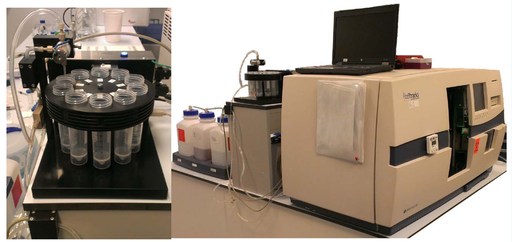

Figure 4: The automated station for the collection of discarded blood samples from the CellSearch AutoPrep waste line.

	SKBR3 (n=6)	T24(n=6)
Recovery Cellsearch	91% ±(7%)	30%±(4%)
Recovery on filter from waste	5% ±(2%)	50% ±(8%)
Cells accounted for*	97% ±(9%)	92%±(12%)

Table 1: Average recovery of 300 spiked EpCAM positive (SKBR3) and negative (T24) cells. Carry over between samples collected in waste was <0.9% ±(0.4%) . The average recovery of the cell lines on the filter from whole blood is 29%±(6%) for COLO320 and 80% ±(16%) for SKBR3 and T24. *Total % of cells found is corrected for filtration loss.

Sample processing

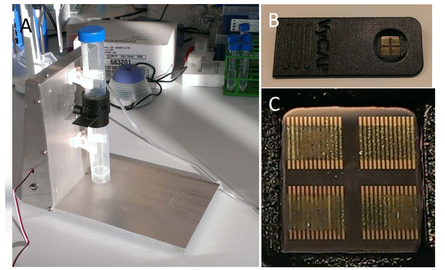

Figure 5: Collected Samples are filtered using a filtration station (A) which provides a fixed pressure across a silicon nitride filter mounted in a slide (B) with >100.000 5um pores (C).

Pressure & recovery

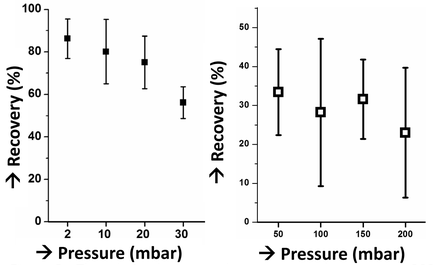

Figure 6: The relation between the pressure and recovery of 300 SKBR3 cells from 1 ml of unfixed whole blood using tracketched membranes (A) and 250 colo320 cells from 7.5 ml of cellsave fixed blood(B).

Using correct pressure is important for CTC recovery [2]. The recovery of the colo320 cell line increased but the drop at higher pressures is less notable.

Staining on Filter

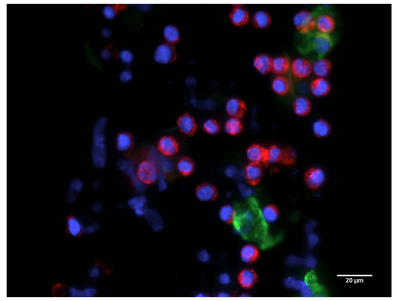

Figure 7: CK positive cells obtained by filtration of blood discarded by the CellSearch AutoPrep from a prostate cancer patient. Stained on filter with CD45-PE, CK-FITC and Hoechst.

Conclusion

We present a method and device that enables the identification and characterization of CTC not detected by the CellSearch system which allows a systematic evaluation of the clinical relevance of these CTC

References

1. Coumans FAW, van Dalum G, Beck M, Terstappen LWMM. Filter characteristics influencing circulating tumor cell enrichment from whole blood. PLoS One. (in press)
2. Coumans FAW, van Dalum G, Beck M, Terstappen LWMM. Filtration parameters influencing circulating tumor cell enrichment from whole blood. PLoS One. (in press)

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