# Isolation of CTC from Diagnostic LeukApheresis


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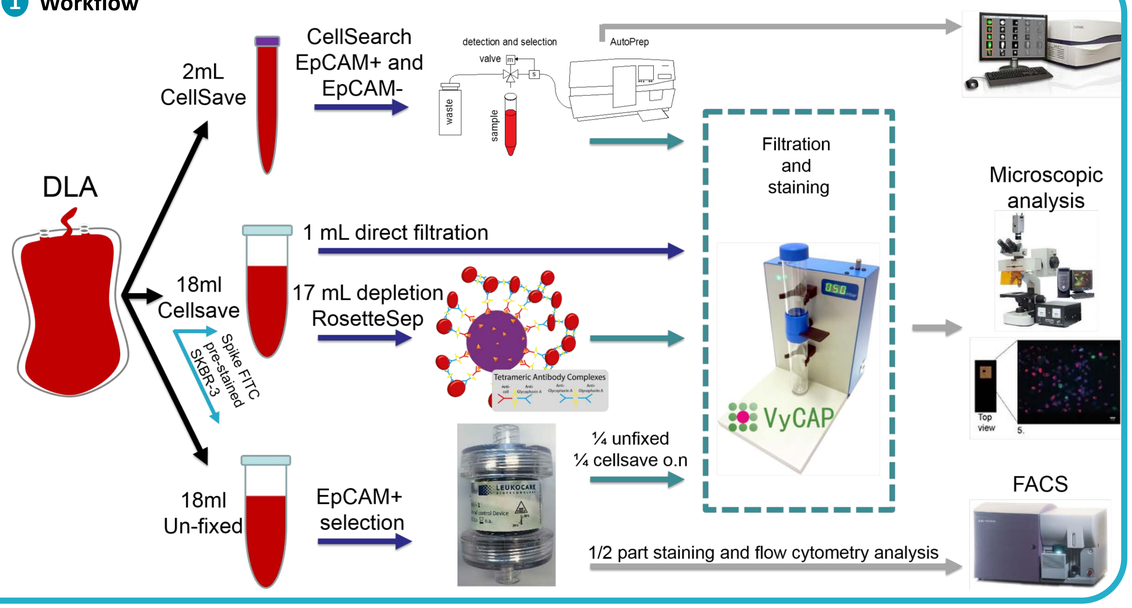
**Introduction:** Circulating tumor cells (CTC) are tumor cells that detach from their primary site, enter the circulatory system, migrate through the body and form secondary tumors at distant sites during the process of cancer metastasis. Peripheral blood represents a minimally invasive source of spreading tumor cells and could be used as a liquid biopsy for diagnosis and to monitor treatment and patient outcome. At present, the CellSearch system is the only validated method for the detection of CTC that has been cleared by the U.S. Food and Drug Administration. This system, designed for the enumeration of CTC in 7.5 mL of blood, detects CTC based on their expression of the epithelial cell adhesion molecule (EpCAM) and cytokeratins. However, the number of CTC that are detected in patients with metastatic carcinomas is in most cases too small to reliably determine tumor heterogeneity and to be representative as a 'liquid biopsy'. Approaches to increase the blood volume to be analyzed are necessary to be able to detect more CTC, and make analysis of heterogeneity between CTC more reliable.

**Methods:**
Diagnostic leukapheresis (DLA) was performed for ~1 hour to obtain 40 mL of product containing ~4 x$10^9$ mononuclear cells representing ~1 liter of blood. Using CellSearch 2 mL of DLA could be processed for EpCAM+ CTC[1] and EpCAM- CTC[2]. Using filtration through microsieves with 5 µm pores a maximum of 1.0 mL of DLA could be processed. To process 18 mL DLA product protocols were developed for leukocyte depletion using RosetteSep™ (StemCell Technologies, USA) and for EpCAM selection using an anti-EpCAM coated column (Leukocare AG). All enriched cell fractions were stained using CD45 PerCP, Cytokeratins PE and the nuclear dye DAPI, followed by fluorescence microscopy scanning and analysis.

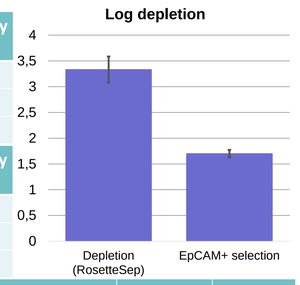
## 1 Workflow



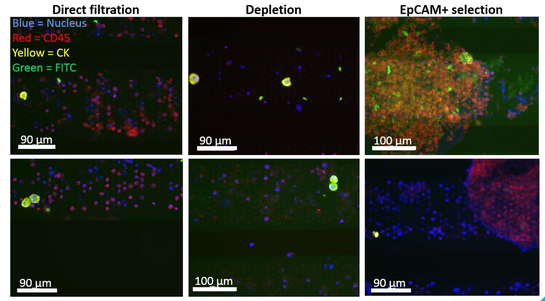

## 2 Results

| | Sample | WBC (x$10^6$/mL) | Volume (mL) | Spiked SKBR-3 | CTC count sieve | Recovery |
|---|---|---|---|---|---|---|
| Direct filtration | 1 | 53 | 1 | 0 | 0 | n/a |
| | 2 | 53 | 1 | 0 | 0 | n/a |
| | 3 | 20 | 1 | 16 | 10 | **62%** |
| Depletion | 1 | 53 | 17 | 0 | 0 | n/a |
| | 2 | 53 | 17 | 0 | 2 | n/a |
| | 3 | 20 | 18 | 280 | 60 | **21%** |

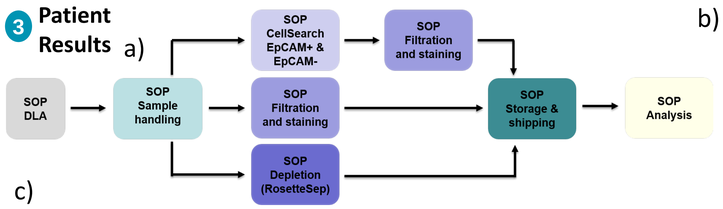
| | Sample | WBC (x$10^6$/mL) | Volume (mL) | Spiked SKBR-3 | CTC count sieve (un-fixed) | CTC count sieve (Cellsave) | CTC count FACS | Recovery |
|---|---|---|---|---|---|---|---|---|
| EpCAM + selection | 1 | 53 | 18 | 0 | 0 | n/a | 0 | n/a |
| | 2 | 53 | 18 | 0 | 0 | 1 | 0 | n/a |
| | 3 | 20 | 18 | 309 | 2 | 1 | 4 | **2%** |




*Tables show results of 3 experiments where direct filtration, depletion and EpCAM+ selection were compared for the isolation of CTC from DLA of which one sample was spiked with SKBR-3 cells.*
*Column chart shows depletion rate after depletion by RosetteSep (n=3, 3.3 log) and EpCAM selection (n=3, 1.7 log). Depletion for RosetteSep was tested in a separate experiment using DLA spiked with LNCaP cells: a recovery of 40 – 60% was measured.*




## 3 Patient Results

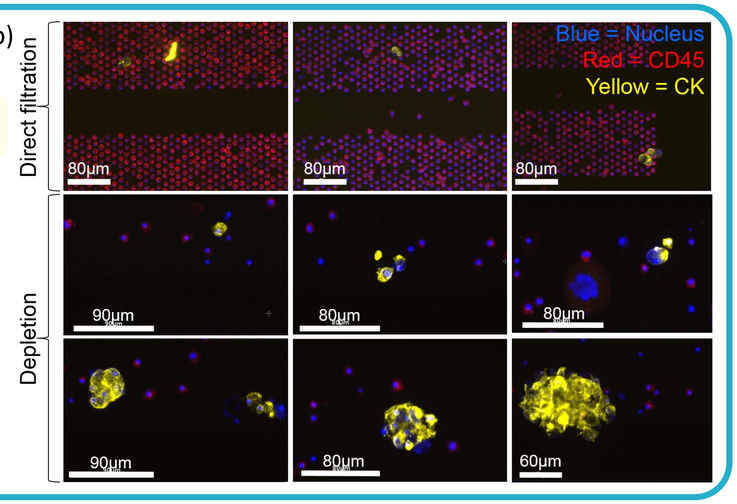



c)

| Sample #* | CellSearch PB # CTC / 7.5mL | CellSearch DLA # CTC / 2x$10^8$ MNC | Depletion & Filtration DLA # CTC / 18mL | Direct filtration DLA # CTC / 5x$10^7$ MNC |
|---|---|---|---|---|
| 1 | 18 | 230 (240 = 96%) | 1 (996 =0.001%) | 2 (60=3.3%) |
| 2 | 15 | 139 (200 = 70%) | 178 (1003 = 18%) | 12 (50=24%) |
| 3 | 13 | 60 (173 = 35%) | 0 (421 = 0%) | 0 (43 = 0%) |
| 4 | 129 | 417 (1720 = 24%) | 30 (5418 = 0.5%) | 4 (430 = 0.9%) |

* Samples kindly provided by Johann de Bono from The Institute of Cancer Research: Royal Cancer Hospital, London.

b)




*a) Workflow used to process prostate patient samples.*
*b) Image gallery with typical images of samples after depletion, filtration and staining c) Table with results of first 4 patient samples processed.*

**Conclusions** Standard operating procedures were developed to isolate CTC in DLA's from breast, prostate and lung cancer patients for evaluation and comparison in the EU sponsored consortiums CTCTrap (www.utwente.nl/tnw/ctctrap/) and CANCER-ID (www.CANCER-ID.eu). Isolation of CTC using the anti-EpCAM coated columns will need further optimization before it can proceed to multicenter comparison. Other methods are currently being evaluated using patient samples.


**References**
[1] J. C. Fisher, et al., pnas, 110 (41), 16580–16585 (2013)
[2] S. de Wit, et al., Sci Rep, 5, 12270 (2015)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