# Maximizing clinical utility of liquid biopsy utilizing a single blood sample for ctDNA and CTC analysis using the Parsortix® System

#40

*Mariacristina Ciccioli[1], Manilyn Teo[1], Amy Templeman[1], David Greaves[1], Aaron Cottingham[1], Anne-Sophie Pailhes-Jimenez[1]*

*[1]ANGLE Europe Limited, 10 Nugent Road, Surrey Research Park, Guildford, Surrey GU2 7AF United Kingdom*

## Introduction

Liquid biopsy has the potential to repeatedly characterize disease in real time at genetic, transcriptional, and protein levels; something not achievable with often a static, single-timepoint tissue biopsy. Various assays (**Figure 1**) have been developed to investigate circulating tumor DNA (ctDNA) in the plasma component of blood, but these assays do not incorporate the cellular component.

Analysis of circulating tumor cells (CTCs) can provide valuable information in addition to degraded ctDNA fragments released from cancer cells. In this study, we developed a method for CTC analysis using the Parsortix® Research System from a single blood tube after plasma was removed for ctDNA analysis.

**Research Use Only. Not for use in diagnostic procedures.**

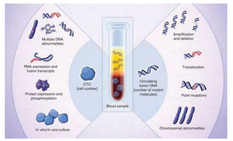


**Figure 1. Circulating biomarkers.** The plasma component (yellow) of a blood sample can be separated by centrifugation from the cellular fraction (red). ctDNA can be detected in the plasma, while CTCs are present in the cellular fraction. Figure adapted from Haber & Velculescu, *Cancer Discovery* 2014; 4:650-61.

## Workflow

The workflow of the assay is illustrated in **Figure 2**. In summary, the study compared detection of cancer cell lines and CTCs in blood samples processed on Parsortix® Research systems with or without plasma removal.

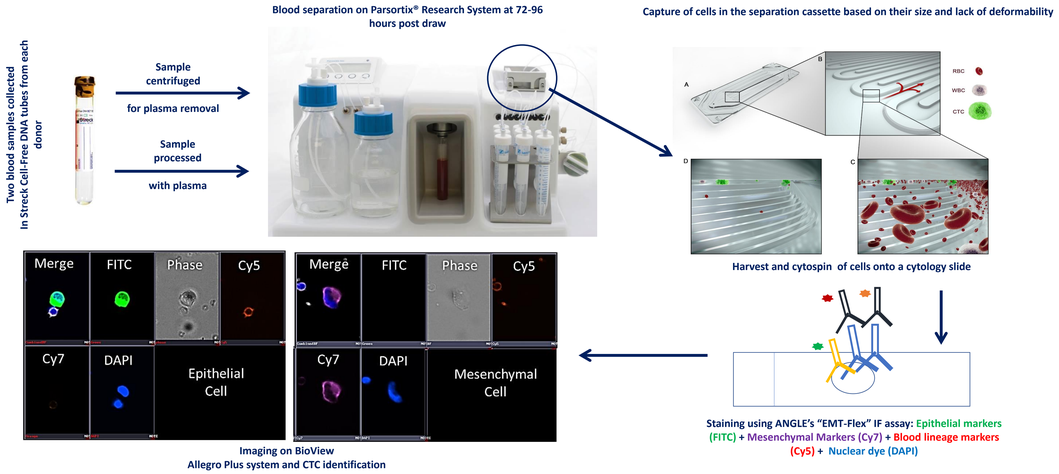


**Figure 2. Schematic representation of the assay workflow.** Two blood samples (8–10 mL) were collected from each set of donors using Streck Cell-Free DNA tubes. Plasma was removed by centrifugation (1600 *g*, 10 minutes) of one sample per donor, whilst the other sample was not centrifuged. The cellular component left in each centrifuged tube (buffy coat and erythrocytes) was resuspended in PBS. All samples were processed on the Parsortix® Research system 72-96 hours post-draw. Harvests were spun onto a positively charged slide (or alternatively collected into wells) and stained using an optimized immunofluorescence panel for detection of epithelial and mesenchymal markers called "EMT-Flex" assay. Slides were analysed using a BioView Allegro Plus automated imaging system. CTCs/cancer cell lines were defined as epithelial (FITC+, Cy7-, Cy5-, DAPI+), mesenchymal (FITC-, Cy7+, Cy5-, DAPI+), or Epithelial-to-Mesenchymal transitioning (FITC+, Cy7+, Cy5-, DAPI+).

The following samples were used:

- ❑ Healthy volunteers (HVs) blood samples spiked with SKBR3 (epithelial) and Hs 578T (mesenchymal) cancer cell lines and harvested into wells or slides for downstream analysis.
- ❑ Samples from Metastatic Breast Cancer (MBC) patients harvested into positively charged slides for downstream IF staining using ANGLE's "EMT-Flex" assay for detection of CTCs.

## Results

**Figure 3** details results obtained from the set of spiked HV samples harvested into wells. No statistically significant difference was observed in the number of cells captured in ANGLE's Parsortix separation cassette (capture percentage) or the number of cells harvested into a well (harvest percentage) between samples processed with or without plasma. Results were comparable between epithelial (SKBR3) and mesenchymal-like cancer cell lines (Hs 578T).

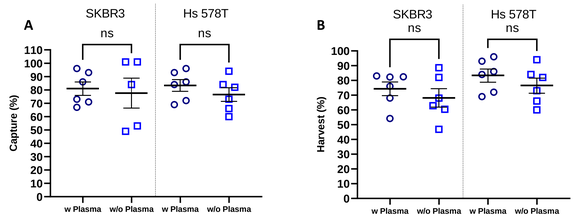


**Figure 3. Epithelial and Mesenchymal cancer cell lines capture and harvest (in wells) with (w) and without (w/o) plasma.** Dot plots show the mean ± Standard Error of the Mean (SEM) of the percentage of **A)** SKBR3 and Hs 578T cells captured and **B)** SKBR3 and Hs 578T cells harvested. No statistically significant difference was observed, *ns= p≥0.05, Paired T-test.*

**Figure 4** details results obtained from the set of spiked HV samples harvested and IF stained using ANGLE's "EMT-Flex" panel for CTC detection. Similarly, no statistically significant difference was observed in the number of cells harvested on slide between samples processed with or without plasma. Cancer cell lines on slides were identified based on their staining phenotype (SKBR3 cells were DAPI+, FITC+, Cy5- and Cy7- and Hs 578T cells were DAPI+, FITC-, Cy5- and Cy7+). Additionally, no qualitative differences were observed in the slides produced for each conditions.

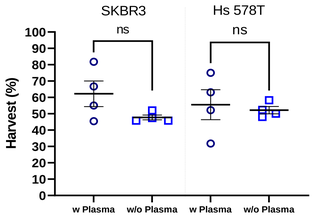


**Figure 4. Epithelial and Mesenchymal cancer cell lines harvest (in Harvest Slides) with (w) and without (w/o) plasma.** Dot plots show the mean ± SEM of the percentage of SKBR3 and Hs 578T cells harvested and IF stained. No statistically significant difference was observed, *ns= p≥0.05, Paired T-test.*

**Figure 5** details results obtained from the set of metastatic breast cancer patients. CTCs were identified in 39% (5/13) of the patients' samples with matched findings in both conditions (w plasma: range = 0 – 36, mean = 7; w/o plasma: range = 0 – 36, mean = 5). The number and size of CTC clusters were comparable between conditions, with ≥1 cluster found in 39% of the samples processed with plasma and 31% of the samples without. No statistically significant difference was observed in CTC and CTC clusters detection between conditions.


| C | w Plasma | w/o Plasma |
|---|---|---|
| N | 13 | 13 |
| ≥1 CTC (%) | 39% | 39% |
| CTC Range (in positive donors) | 4-36 | 4-36 |
| CTC mean (in positive donors) | 17 | 14 |
| CTC median (in positive donors) | 13 | 10 |
| Donor with ≥1 CTC Cluster (%) | 39% | 31% |
| N Cluster per donor (positive donors) | 1-7 | 2-4 |
| Range CTC per cluster | 2-13 | 2-13 |

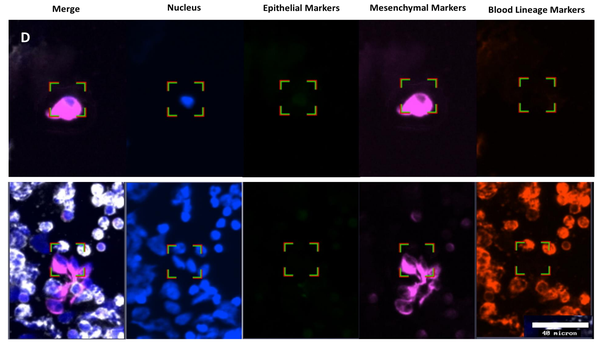


**Figure 5. CTC detection in MBC patients blood samples processed with or without plasma.** Dot plot shows mean ± SEM of the number of **A)** CTCs and **B)** CTC clusters identified in each donor per condition. No statistically significant difference was observed, *ns= p≥0.05, Paired T-test.* **C)** Table showing number of donors included in each cohort (N), percentage (%) of donors with ≥1 CTC, range, mean and median of CTCs captured within the positive group, percentage of donors with ≥1 CTC cluster, range of CTC clusters per donor, and range of number of CTCs per cluster; **D)** Representative images of a single mesenchymal CTC (top) and a cluster of mesenchymal CTCs (bottom). Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in red or white in the merge, Nucleus (DAPI) in blue.

## Conclusions

This study demonstrated that no cancer cell / CTC loss occurs when pre-processing blood samples by centrifugation and plasma removal before separating the sample on Parsortix systems.

ANGLE's workflow allows combining CTC isolation using the Parsortix® system with plasma removal for subsequent ctDNA analysis from a single tube of blood, opening the possibility for clinical laboratories to broaden the potential clinical utility of their assays and limiting the overall blood volume requirement. Additionally, ANGLE's workflow has the added advantage of enabling the processing of blood up to 144 hours post collection, allowing the shipment of samples for centralised analysis in support of global clinical trials.