A highly sensitive method for circulating tumour cell antigen quantification in liquid biopsy
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Introduction
Circulating tumour cells (CTCs) have the potential to be used as non-invasive biomarkers to aid detection, diagnosis and management of cancer. Currently, immunofluorescence (IF) staining is a standard technique to identify CTCs. However, this can be limited by difficulty with sensitivity, specificity and subjective interpretation of antibody staining.

Aim
To develop an automated highly sensitive method for antigen quantification of CTCs captured using Parsortix™ cell separation system.

Materials and Methods
CTCs can be captured from peripheral blood and harvested using Parsortix (Figure 1). MESO™QuickPlex™ SQ120 instrument enables electrochemiluminescence detection and quantification of proteins.

Figure 1: The Parsortix cell separation system isolates CTCs from whole blood. The instrument consists of the cell separation cassette held by a clamp through which blood flows and is separated on the basis of size and compressibility.

Results
Parsortix™ recovers 75% SKBR3 cells and 60% SKMEL28 cells from 10 ml EDTA blood samples. There is a residual background of ~5000 leukocytes. This varies according to donor.

Cells recovered using Parsortix are suitable for antigen quantification using the Meso instrument. The assay is highly sensitive, specifically detecting antigen in as low as 14 target cells, with no background detection of leukocytes from healthy normal volunteers. The ErbB2 assay gave a larger signal window than the gp100 assay.

Figure 2: SKBR3 and SKMEL28 cells were spiked (~200 cells) into whole blood and harvested using Parsortix. Both target cells (A) and residual leukocytes (B) were enumerated.

Figure 3: SKBR3 cells were spiked into whole blood, separated and automatically stained for ErbB2 before imaging using LeicaDMI6000. ErbB2 stained SKBR3 cells are caught on the separating structure, with one leukocyte (CD45 positive) cell remaining.

Figure 4: A range of cell lysates were prepared containing equivalent of 10,000 down to 14 cells. In addition, unspiked and spiked blood was separated on Parsortix and lysates prepared. ErbB2 detection antibody was used for SKBR3 cells (A); gp100 detection antibody was used for SKMEL28 cells (B).

The specificity and sensitivity of antigen detection in CTCs achieved with this combination of Parsortix and Meso QuickPlex suggests substantial potential for the method in clinical liquid biopsies.