CellKeep™ slide enhances retention of circulating tumour cells harvested from patient blood samples using the Parsortix® system

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Introduction

Circulating tumour cells (CTCs) are rare, therefore efficient harvest and deposition onto slides of CTCs from patient blood samples is paramount. However, industry standard microscopy slides such as Cytoslides, incur significant cell loss when used for sample processing downstream of CTC isolation using a Parsortix® instrument. To address this, ANGLE have developed the CellKeep™ slide.

CellKeep slides consist of a plastic loading chamber attached to a glass slide (**Figure 1A**). The Parsortix harvest is collected directly into the CellKeep slide through the funnel of the chamber (**Figure 1B**), and the device centrifuged. A wicking cap is used to remove the excess liquid after the first centrifugation (**Figure 1C**), then drying buffer is added and the CellKeep slide is centrifuged again. Another wicking cap is used to remove the drying buffer, after which the funnel assembly is removed (**Figure 1D**). The slide is then dried for one hour at 37°C before fixation and immunofluorescence (IF) staining. This study compared the performance of CellKeep slides to Cytoslides.

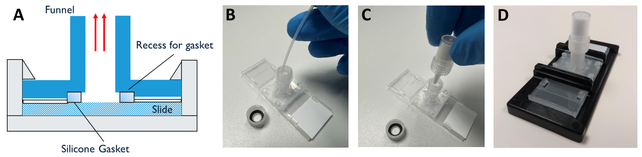

Figure 1. Representative images of the CellKeep Slide. Schematic diagram **(A).** Harvesting from Parsortix into the CellKeep funnel **(B)**. Insertion of wicking cap into the CellKeep funnel **(C).** CellKeep slide inserted into detacher tool **(D)**.

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Workflow

Peripheral blood from Healthy Volunteers (HVs) was drawn into Streck Cell-Free DNA Blood Collection tubes (BCTs). At 96 hours post draw, blood was spiked with epithelial marker positive (HCC1954) and mesenchymal marker positive (Hs578T) breast cancer cell lines, then processed with a Parsortix® instrument. Harvests were cytocentrifuged onto ANGLE CellKeep slides or Cytoslides then stained using ANGLE's Portrait® Flex IF assay for the detection of epithelial and mesenchymal markers, and PBMC exclusion markers. The same workflow was then used to process blood from a cohort of 11 metastatic breast cancer (MBC) patients and 12 HVs as a control group (all unspiked) within 144 hours of collection. All stained slides were imaged using a BioView Allegro Plus imaging system.

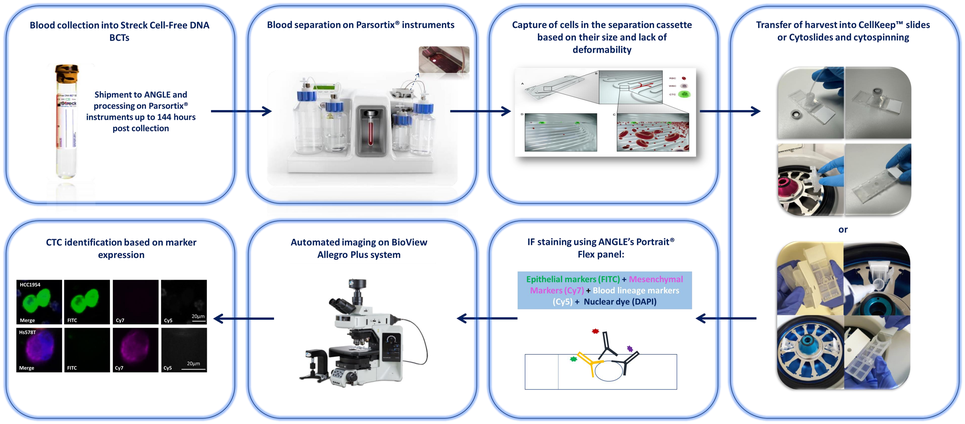


Figure 2. Schematic representation of the assay workflow. Peripheral blood was drawn into Streck Cell-Free DNA tubes and stored for up to 144 hours from collection before processing. Blood samples were processed on the Parsortix® instrument, a microfluidic device capable of capturing and harvesting CTCs from bodily fluids based on cell size and lack of deformability. Harvested CTCs were cytospun onto ANGLE's CellKeep™ slides or Cytoslides. Slides were stained using ANGLE's IF-based Portrait® Flex assay, comprising a nuclear dye (Hoechst) and antibodies against epithelial markers (FITC), mesenchymal markers (Cy7), and blood lineage markers (Cy5), including antigens expressed by blood cells (lymphocytes, macrophages, granulocytes, monocytes, fibroblasts, and cells of megakaryoblastic potential). When staining with the Portrait® Flex assay, the Cy3 channel is left intentionally blank to allow the integration of antibodies targeting an additional marker of interest (for example PD-L1 or HER2) to the assay. Stained slides were imaged using a BioView Allegro Plus system, a platform equipped with artificial intelligence for automated imaging, CTC candidate identification and reporting.

Analytical results

- Harvest linearity in each support was assessed by plotting the number of cells harvested against the number of cells spiked across five spiking levels.
  - CellKeep slides demonstrated greater harvest linearity than Cytoslides over a range of 0 - 500 spiked cells. HCC1954: CellKeep R^2=0.94, Cytoslide R^2=0.46 (**Figure 3A-B**); Hs578T: CellKeep R^2=0.92, Cytoslide R^2=0.53 (**Figure 3C-D**).
- Percentage spiked cell line harvest (accuracy) for each support was assessed by normalising the number of cells harvested and stained with the number of cells spiked for each sample.
  - Mean percentage harvest of both cancer cell lines was significantly higher with CellKeep slides; HCC1954: CellKeep 41.9%, Cytoslide 10.9% (p<0.0001); Hs578T: CellKeep 40.5%, Cytoslide 5.9% (p<0.0001) (**Figure 3E**).
  - Mean total nucleated cells per sample was also significantly higher with CellKeep slides (14570) compared to Cytoslides (6189) (p<0.0001) (**Figure 3F**).
- IF staining quality was comparable between CellKeep slides and Cytoslides (**Figure 3G**).

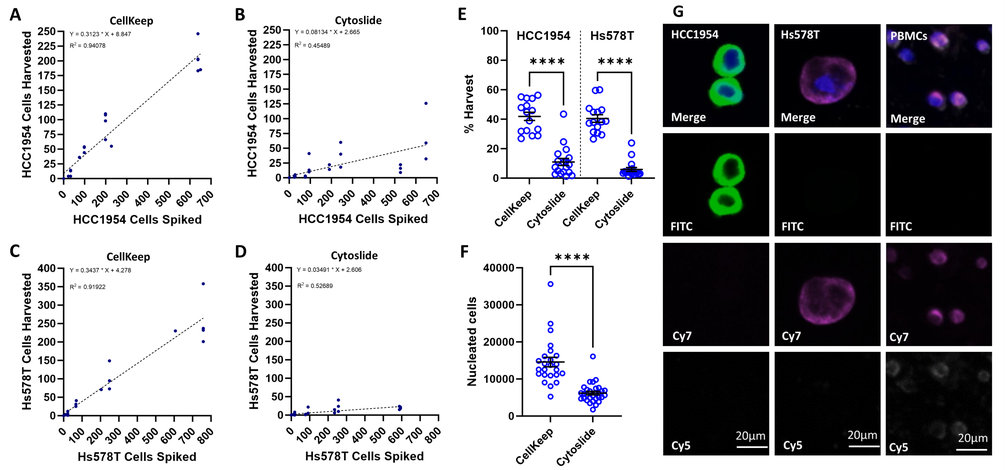


Figure 3. Analytical performance Data. Dot plots showing the number of cancer cells spiked (x-axis) vs the number of cancer cells harvested (y-axis) across spiking levels for HCC1954 cells harvested onto **(A)** CellKeep Slides and **(B)** Cytoslides; and Hs578T cells harvested onto **(C)** CellKeep Slides and **(D)** Cytoslides. The trendline equations and R^2 values are included for the first order polynomials. **(E)** Dot plot showing mean ± 1 SEM of the percentage retention of spiked cancer cell lines (final cell count / spiked cell count) × 100)(Kruskal-Wallis test, *p**** <0.0001)*; **(F)** Dot plot showing mean ± 1 SEM of the total number of nucleated cells detected in each sample (Mann-Whitney test, *p**** <0.0001)*; **(G)** Representative images of cancer cell lines and PBMCs on CellKeep slides after IF staining with the Portrait Flex assay. (Left= HCC1954; Centre= Hs578T Right= PBMCs; From top to bottom: Merge, epithelial markers in green (FITC), mesenchymal markers in purple (Cy7), blood lineage markers in white (Cy5)). Micron bar = 20 µM.

Patients' results

A

	CellKeep	Cytoslide
N	11	11
N donors with ≥1 CTC (CTC+)	6 (55%)	4 (36%)
Total CTCs detected	116	49
Mean CTCs (CTC+ donors)	19	8
Median CTCs (CTC+ donors)	22	7
Range (Total CTCs)	2-37	4-22
Total Single CTCs detected	33	11
Mean Single CTCs (CTC+ donors)	6	2
Median Single CTCs (CTC+ donors)	3	1
Range (Single CTCs)	1-21	1-7
N CTC+ donors with ≥1 CTC Cluster	6 (100%)	4 (100%)
Total CTC Clusters detected	20	11
Mean CTC Clusters	3	1
Median CTC Clusters	3	2
CTCs per Cluster	2-20	2-10
Mean Single Events	8	4
Median Single Events	6	4
Range (Single Events)	1-25	2-8

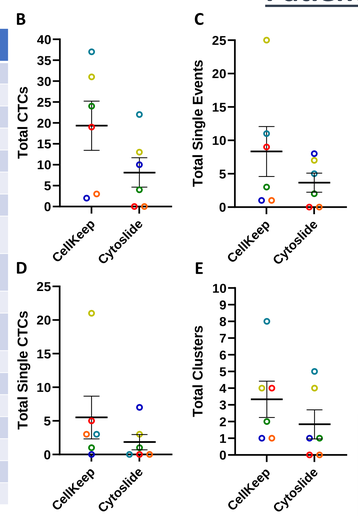
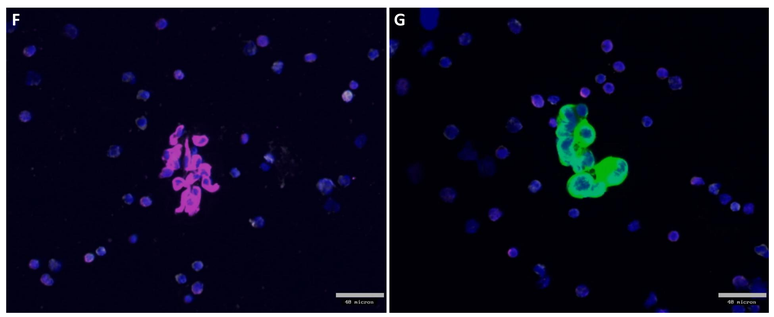


Figure 4. Evaluation of MBC patients' samples separated on Parsortix® instruments. (A) Table showing number (N) of donors in each group, percentage of CTC+ donors (≥1 CTC); mean, median, and range of total CTCs, total single CTCs, total CTC clusters, and total single events (single CTCs plus clusters as one event). **(B)** Dot plot showing mean ±1 SEM of the total number of CTCs isolated in MBC patients. (In all graphs colour coding of data points denotes matched samples from the same donor; No significant differences were detected (Mann-Whitney Test P≤0.05)). **(C)** Dot plot showing mean ±1 SEM of the total number of single CTCs isolated in MBC patients. **(D)** Dot plot showing mean ±1 SEM of the total number of CTC clusters isolated in MBC patients. **(E)** Dot plot showing mean ±1 SEM of the total number of Single Events. **(F)** Representative image of a cluster of mesenchymal CTCs on CellKeep Slide (Micron bar = 40 µm). **(G)** Representative image of a cluster of epithelial CTCs on CellKeep slide (Micron bar = 40 µm). DNA in blue (DAPI), epithelial markers in green (FITC), mesenchymal markers in purple (Cy7), blood lineage markers in white (Cy5).

- Samples from 11 MBC patients, and 12 HVs as a control group, were processed as per **Workflow Section**.
- Patient CTC positivity rate (≥1 CTC detected) was higher with CellKeep slides compared to Cytoslides (**Figure 4A**), with only 67% (4/6) of patients which presented CTCs in CellKeep slides also presenting CTCs in Cytoslides.
- Overall number of CTCs (mean, median and range) per sample was higher with CellKeep slides compared to Cytoslides (**Figure 4B**).
- Overall number of single events (single CTCs plus each cluster as one event) per sample was also higher with CellKeep slides compared to Cytoslides (**Figure 4C**).
- With both slide types all CTC+ samples had ≥1 CTC cluster detected, however the number of clusters detected (mean and median), and the size range of detected clusters was higher with CellKeep slides compared to Cytoslides (**Figure 4E**).
- No significant difference between slide types was detected (**Figure 4B-E**).
- No difference in the phenotype of CTCs were observed between slide types.
- No CTCs (or CTC-like cells) were observed in any HV samples.



Conclusions

The ANGLE CellKeep™ slides outperformed Cytoslides in all tested parameters with contrived samples (percentage harvest (accuracy), linearity, total nucleated cell capture) and patient derived samples (positivity rate, overall CTCs numbers, capture and preservation of CTC clusters morphology). The data gathered in this study suggests that combining CellKeep slides with the Parsortix system boosts the potential utility of every blood sample processed.