Supplementary Materials

Methods

Cell line maintenance

The SKBR3 cell line was maintained in McCoy's 5a medium with L-glutamine (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (Sigma Aldrich) and 1% Penicillin-Streptomycin (PS) (Sigma Aldrich), the MCF7 cell line in EMEM (ATCC) supplemented with 10% FBS, 0.01 mg/mL human insulin (Merck) and 1% PS, and the Hs578T cell line in DMEM with L-glutamine (Sigma Aldrich) supplemented with 10% FBS, 0.01 mg/mL bovine insulin (Sigma Aldrich) and 1% PS.

Additional precision studies

The additional precision studies were conducted in accordance with the CLSI guideline *EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition*. Imprecision estimates for the Parsortix® PC1 System were evaluated for the following:

- Live vs fixed cells using Precision Tubes or ~20 live SKBR3 cells spiked into blood collected from HVs: For live SKBR3 cells, ~20 live, pre-labelled SKBR3 cells were directly spiked into 7.5mL aliquots of blood. For fixed SKBR3 cells, Precision Tubes were spiked into ~10mL blood samples. For both fixed and live cells, data was obtained from a total of 400 samples processed on 10 Parsortix® PC1 Systems, two runs per system per day, over a total of 20 non-consecutive days.

- Different cell separation cassette lots using Precision Tubes spiked into 2.5mL aliquots of PBS: Data was obtained from a total of 200 samples processed on 10 Parsortix® PC1 Systems, two runs per system per day per cassette lot, over a total of 10 non-consecutive days.
- Different laboratories using Precision Tubes spiked into 2.5mL aliquots of PBS: The ANGLE R&D Laboratory, a research laboratory located at the MD Anderson Cancer Center (MDA) in Houston, TX, USA, and a research laboratory located at the University of Rochester Medical Center (URMC), in Rochester, NY, USA prepared and processed samples. Data was obtained from a total of 800 samples processed on 20 Parsortix® PC1 Systems, two runs per system per day, over a total of 20 non-consecutive days for each laboratory site (ANGLE R&D: 10 systems, MDA: six systems, and URMC: four systems).

Data analysis

Linearity: The sample size was determined and the data was evaluated in accordance with the methods described in the CLSI (formerly NCCLS) guideline *EP06-A: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach.* A minimum of 10 spiked samples were processed for each spiking level and cell line. For each spiked sample, the percentage of spiked cells harvested was calculated by dividing the observed number of cells in each harvest by the number of cells spiked into each sample. For each spike level, the average (mean), standard deviation (SD) and percent coefficient of variation (%CV) were calculated for the actual number of cells spiked, the number of cells harvested, and the percent harvest results. An overall average harvest rate and 95% confidence

intervals (95% CI) was also determined for each cell line. The repeatability SD at each spike level for each individual replicate was determined by calculating the difference between the actual number of cells harvested and the average number of cells harvested (difference from average) and then squaring this value and dividing it by 2 (repeatability SD). The percentage difference SD at each spike level for each individual replicate was determined by dividing the difference from average by the average number of cells harvested (% difference from average) and then squaring this value and dividing it by 2 (percentage difference SD). The averages and maximums of the individual repeatability SD results were determined at each spike level for each cell line. All of these calculations were performed separately for each cell line and condition (*i.e.,* live or fixed).

The linearity of each cell line and condition were evaluated separately using Microsoft® Excel 2016 and Analyse-It® Ultimate Edition for Microsoft® Excel, v5.65.7; data for the actual number of cells spiked was plotted on the X-axis and the number of cells harvested was plotted on the Y-axis (10 replicate results for each cell spike level). Polynomial regression analyses for the first, second and third-order polynomials were conducted, and the model constants and regression coefficients for each model were determined and compared.

Limit of Blank (LoB) and Limit of Detection (LoD): The sample sizes for the LoB and LoD studies were selected based on the nonparametric data analysis methods described in the CLSI guideline *EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*. A minimum of 60 replicates for unspiked (blank) samples with evaluable harvest results and a minimum of 60 replicates for each level of spiked samples with evaluable harvest results were used to non-parametrically determine the LoB and

LoD with a type I error level (α = false positive rate) of 5% and a type II error level (β = false positive rate) of 5% (*i.e.,* power of 95%). The LoB was taken as the value of the blank sample result at the rank position corresponding to the 95th percentile for the distribution of the blank sample results. The LoD was defined as the minimum number of live cells required to be present in a blood sample in order for at least one cell to be harvested by the Parsortix® PC1 System ≥95% of the time.

Precision: The sample sizes and analyses of the data from the various precision studies were determined and performed in accordance with the methods described in the CLSI guideline *EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition*. The percentage of cells harvested for each precision sample was calculated by dividing the number of cells observed in each harvest by the number of cells spiked into each sample. The statistical analyses were performed using Microsoft® Excel 2016 and Analyse-It® Ultimate Edition for Microsoft® Excel (v5.66 or higher). For each individual precision study, the factors of day and run were used in two-factor ANOVA models to calculate the repeatability (within-run) and reproducibility (within-laboratory) imprecision estimates (%CV) for the Parsortix® PC1 System. Additionally, three-factor ANOVA models were used to calculate the repeatability and reproducibility imprecision estimates for all combined data and between-site, between-cassette, and between-cell (live and fixed) imprecision estimates.

Reproducibility: To determine the sample size required to estimate the mean percentage of spiked tumor cells harvested from MBC patient and HV subject blood samples using a 95% confidence interval with a margin of error of ± 5%, the following formula was used:

$$n = \left(\frac{Z\sigma}{E}\right)^2$$

where *Z* is the value from the standard normal distribution reflecting the confidence level that will be used (*e.g., Z* = 1.96 for 95%), *σ* is the standard deviation of the outcome variable and *E* is the desired margin of error (*e.g.,* confidence interval). The formula above generates the minimum number of subjects required to ensure that the margin of error in the confidence interval for the mean does not exceed *E*.

Assuming a standard deviation (*σ*) of ~22% for the average % harvest, a 95% confidence interval extending ± 5% (*e.g.,* margin of error [*E*] = 5%) from the observed average % harvest would require a sample size (*n*) of 75 samples. The percentage of spiked, pre-labelled cells harvested from each sample was calculated by dividing the number of pre-labelled cells observed in each harvest by the actual number of cells spiked into each sample. For both MBC patients and HV subjects, the average (mean), SD, 95% CI and median percent harvest were calculated.

Additional Results

Precision

Additional precision studies to assess the ability of the Parsortix® PC1 System to harvest live and fixed SKBR3 cells spiked into blood samples were performed at the ANGLE R&D laboratory. Separate 20-day precision studies using 400 contrived samples each consisting of ~20 live or fixed pre-labelled SKBR3 cells spiked into 7.5mL and 10mL of blood, respectively, were processed using 10 different Parsortix® PC1 Systems for each study. The overall average harvest rate for live SKBR3 cells spiked into 7.5mL of blood was 70.4%, compared to an overall harvest rate of 89.4% for fixed SKBR3 cells spiked into ~10mL of blood. The lower average harvest of live

cells was expected due to their greater deformability compared to the more rigid fixed cells, which also resulted in higher imprecision estimates for the harvesting of live cells compared to the harvesting of fixed cells. The repeatability (within-run) and reproducibility (withing-laboratory) %CV estimates for live cells were 21.1% (95% CI = 19.6% - 22.7%) and 22.0% (95% CI = 20.6% - 23.9%), respectively, compared to 10.2% (95% CI = 9.5% - 11.0%) and 10.3% (95% CI = 9.7% - 11.2%), respectively, for fixed cells. The imprecision estimates for fixed SKBR3 cells spiked into 10mL of blood were comparable to those for fixed SKBR3 cells spiked into 2.5mL of PBS when processed at the ANGLE R&D laboratory (repeatability %CVs of 10.2% vs 14.0% and reproducibility %CVs of 10.3% vs 14.2%, respectively). The combined repeatability and reproducibility %CV estimates for the ability of the Parsortix® PC1 System to harvest ~20 live or fixed pre-labelled SKBR3 cells spiked into ≥7.5mL of blood were 15.4% (95% CI = 14.6% - 16.3%) and 23.2% (95% CI = 17.5% - 537.8%), respectively, with a between-cell imprecision estimate of 16.8% (95% CI = 7.5% - 537.5%).

Additional 10-day precision studies were performed at the ANGLE R&D laboratory using three different separation cassette manufacturing lots. For each cassette lot, 200 contrived samples consisting of ~20 fixed, pre-labelled SKBR3 cells spiked into 2.5mL of PBS were processed on 10 different Parsortix® PC1 Systems. The overall average harvest rates for the different separation cassette lots ranged from 80.7% to 82.2%. The repeatability (within-run) and reproducibility (within-laboratory) %CV estimates for the different cassette lots ranged from 12.9% to 15.9% and 13.4% to 15.9%, respectively. The combined repeatability and reproducibility %CV estimates for the Parsortix® PC1 System over the three different cassette lots were 14.4% (95% CI = 13.7% - 15.4%) and 14.5% (95% CI = 13.8% - 15.9%), respectively, with

within-cassette and between-cassette imprecision %CV estimates of 14.5% (95% CI = 13.8% - 15.5%) and 0.0% (95% CI = 0.0% - 5.7%), respectively.

Separate 20-day precision studies were performed at three different laboratory sites (URMC, MDA and ANGLE R&D), using contrived samples consisting of ~20 fixed, pre-labelled SKBR3 cells spiked into 2.5mL of PBS. A total of 800 samples were processed at the three different laboratory sites using 20 different Parsortix® PC1 Systems. The overall average harvest rates at the individual sites ranged from 65.4% to 81.6%. The repeatability (within-run) and reproducibility (within-laboratory) %CV estimates at the individual sites ranged from 14.0% to 22.9% and 14.2% to 23.4%, respectively. The combined repeatability and reproducibility %CV estimates for the Parsortix® PC1 System over the three different sites were 17.0% (95% CI = 16.1% – 17.9%) and 20.6% (95% CI = 18.1% - 72.9%), respectively, with within-site and between-site imprecision %CV estimates of 17.3% (95% CI = 16.5% - 18.3%) and 11.2% (95% CI = 5.7% - 70.8%), respectively.

Reproducibility

It was found that two different batches of fixed, pre-labelled SKBR3 cells (lot numbers CELL-311 and CELL-336) were used to make the Precision Tubes used to spike the blood samples during the course of the study. The average percentage harvests for these two lot numbers were found to be significantly different (66.0% ±15.1%, median = 66.7%, for lot number CELL-311; and 77.6% ±12.7%, median = 80.0%, for lot number CELL-336; t-test p-value <0.0001, **Supplementary Figure 1A**).

Further investigation showed that 73.0% of the MBC patients had their blood samples spiked with Precision tubes made using SKBR3 lot number CELL-311

compared to only 57.9% of the HV subjects. **Supplementary Figure 1B** shows a comparison of the percent harvest results between the HV subjects and the MBC patients by the two different SKBR3 lot numbers, confirming that there were no significant differences in the ability of the Parsortix® PC1 System to harvest SKBR3 cells spiked into the blood of HV subjects compared to MBC patients.

Supplementary Tables

Table SI. Accepted cell number ranges for each target cell spike level

Target cell spike level	Accepted cell number range	Study type
1	1	LoD
2	2	LoD & Linearity
3	3	LoD
4	4	LoD
5	5	LoD, Linearity & Precision
10	10	Linearity & Precision
15	15	Linearity
20	18-22	Precision
25	24-26	Linearity
50	48-52	Linearity & Precision
75	72-78	Linearity
100	95-105	Linearity & Precision

LoD: Limit of Detection

Table SII. Percent Harvest Estimates – Live SKBR3 Cells spiked into 7.5mL of blood

Mean # of Cells Spiked (SD, %CV)	Mean # of Cells Harvested (SD, %CV)	Mean Percent Harvest (SD, %CV)	Range of Percent Harvest
2 (0, 0%)	2 (1, 50%)	70% (35%, 50%)	0% to 100%
5 (0, 0%)	4 (2, 50%)	68% (21%, 31%)	40% to 100%
10 (0, 0%)	7 (2, 29%)	66% (19%, 29%)	30% to 90%
15 (0, 0%)	10 (4, 40%)	65% (21%, 32%)	33% to 100%
25 (1, 4%)	19 (4, 21%)	73% (13%, 18%)	48% to 100%
50 (2, 4%)	35 (6, 17%)	69% (10%, 14%)	51% to 81%
76 (2, 3%)	52 (8, 15%)	69% (10%, 14%)	55% to 86%
100 (2, 2%)	69 (16, 23%)	69% (15%, 22%)	40% to 84%
123 (16, 13%)	89 (26, 29%)	74% (23%, 31%)	23% to 103%
232 (14, 6%)	138 (26, 19%)	59% (10%, 17%)	43% to 78%
489 (69, 14%)	331 (60. 18%)	68% (10%, 15%)	52% to 83%
1113 (44, 4%)	729 (81, 11%)	66% (7%, 11%)	55% to 77%
	Overall (2 - ~100 cells)	**69% (19%, 27%)**	**0% to 100%**
	Overall (2 - ~ 1000 cells)	**68% (17%, 25%)**	**0% to 103%**

SD: Standard deviation, %CV: percent coefficient of variation. Percent harvest results of >100% are reflective of the inherent inaccuracies with cultured cell spiking due to the nature of the cultured cells, i.e., clumping, adhesion, etc.

Table SIII. Percent Harvest Estimates – Live MCF7 Cells spiked into 7.5mL of blood

Mean # of Cells Spiked (SD, %CV)	Mean # of Cells Harvested (SD, %CV)	Mean Percent Harvest (SD, %CV)	Range of Percent Harvest
2 (0, 0%)	2 (1, 50%)	85% (41%, 48%)	0% to 150%
5 (0, 0%)	4 (2, 50%)	70% (22%, 31%)	20% to 100%
10 (0, 0%)	8 (2, 25%)	72% (18%, 25%)	50% to 100%
15 (0, 0%)	11 (4, 36%)	71% (24%, 34%)	33% to 107%
25 (1, 4%)	17 (4, 24%)	66% (13%, 20%)	46% to 83%
51 (2, 4%)	35 (6, 17%)	69% (12%, 17%)	51% to 85%
76 (2, 3%)	58 (8, 14%)	77% (11%, 14%)	56% to 93%
100 (4, 4%)	76 (6, 8%)	75% (5%, 7%)	66% to 83%
	Overall	**73% (21%, 28%)**	**0% to 150%**

SD: Standard deviation, %CV: percent coefficient of variation. Percent harvest results of >100% are reflective of the inherent inaccuracies with cultured cell spiking due to the nature of the cultured cells, i.e., clumping, adhesion, etc.

Table SIV. Percent Harvest Estimates – Live Hs578T Cells spiked into 7.5mL of blood

Mean # of Cells Spiked (SD, %CV)	Mean # of Cells Harvested (SD, %CV)	Mean Percent Harvest (SD, %CV)	Range of Percent Harvest
2 (0, 0%)	2 (1, 50%)	90% (32%, 36%)	0% to 100%
5 (0, 0%)	4 (1, 25%)	80% (19%, 24%)	40% to 100%
10 (0, 0%)	8 (3, 38%)	76% (20%, 26%)	60% to 120%
15 (0, 0%)	12 (2, 17%)	76% (13%, 17%)	53% to 93%
26 (1, 4%)	20 (3, 15%)	77% (11%, 14%)	64% to 96%
51 (2, 4%)	41 (5, 12%)	80% (9%, 11%)	62% to 90%
77 (2, 3%)	60 (8, 13%)	78% (9%, 12%)	59% to 94%
100 (4, 4%)	76 (7, 9%)	76% (6%, 8%)	64% to 82%
	Overall	**79% (17%, 21%)**	**0% to 120%**

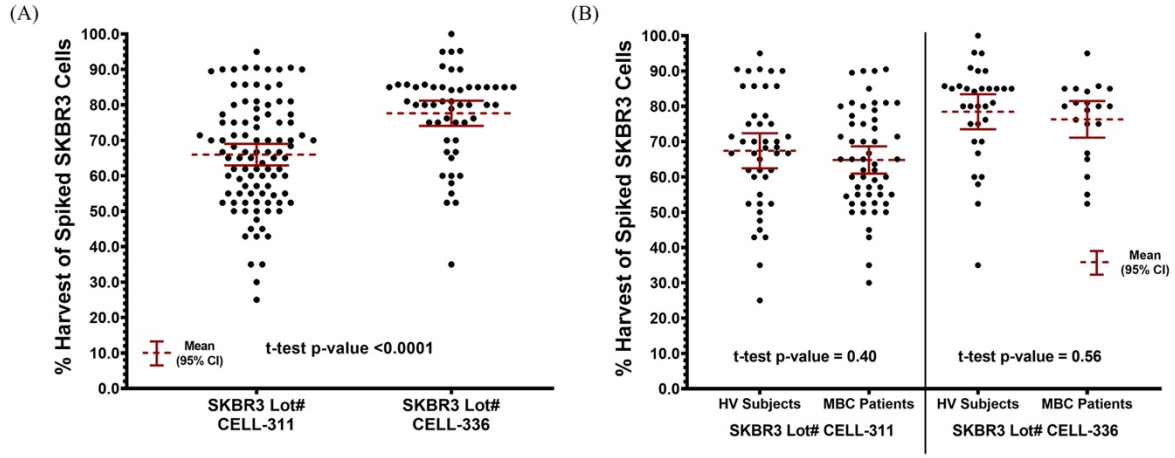
SD: Standard deviation, %CV: percent coefficient of variation. Percent harvest results of >100%are reflective of the inherent inaccuracies with cultured cell spiking due to the nature of the cultured cells, i.e., clumping, adhesion, etc.

Table SV. Percent Harvest Estimates – Fixed SKBR3 Cells spiked into 7.5mL of blood

Mean # of Cells Spiked (SD, %CV)	Mean # of Cells Harvested (SD, %CV)	Mean Percent Harvest (SD, %CV)	Range of Percent Harvest
2 (0, 0%)	2 (1, 50%)	85% (24%, 28%)	50% to 100%
5 (0, 0%)	5 (1, 20%)	86% (16%, 19%)	60% to 100%
10 (0, 0%)	10 (1, 10%)	94% (10%, 11%)	80% to 110%
15(0, 0%)	14 (1, 7%)	91% (6%, 7%)	80% to 100%
25 (1, 4%)	23 (2, 9%)	93% (7%, 8%)	80% to 100%
51 (2, 4%)	46 (4, 9%)	91% (6%, 7%)	82% to 102%
76 (2, 3%)	68 (5, 7%)	89% (5%, 6%)	80% to 96%
101 (3, 3%)	90 (12, 13%)	89% (12%, 13%)	56% to 100%
	Overall	**90% (12%, 14%)**	**50% to 110%**

SD: Standard deviation, %CV: percent coefficient of variation. Percent harvest results of >100% are reflective of the inherent inaccuracies with cultured cell spiking due to the nature of the cultured cells, i.e., clumping, adhesion, etc.

Supplementary Figure 1. Reproducibility of the Parsortix® PC1 System – Fixed SKBR3 Cell Lot Number Investigation.



Plots of the percent harvest results generated from the spiking of HV subject and MBC patient blood samples using different lots of fixed SKBR3 cells: A) In all samples by SKBR3 Cell Lot Number used to make the Precision Tubes used for spiking. B) In HV subjects and MBC patients separately by SKBR3 Cell Lot Number used to make Precision Tubes used for spiking.