

**EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Parsortix® PC1 system (device)**

DECISION SUMMARY

A. DEN Number:

DEN200062

B. Purpose for Submission:

De Novo request for evaluation of the Parsortix® PC1 device

C. Measurands:

Not applicable

D. Type of Test:

Circulating tumor cell enrichment device

E. Applicant:

ANGLE Europe Ltd.

F. Proprietary and Established Names:

Parsortix® PC1 device

G. Regulatory Information:

1. Regulation section:

21 CFR 866.6110

2. Classification:

Class II

3. Product code:

QSA

4. Panel:

H. Indications for use:

1. Indications for use:

The Parsortix[®] PC1 system is an in vitro diagnostic device intended to enrich circulating tumor cells (CTCs) from peripheral blood collected in K₂EDTA tubes from patients diagnosed with metastatic breast cancer. The system employs a microfluidic chamber (a Parsortix cell separation cassette) to capture cells of a certain size and deformability from the population of cells present in blood. The cells retained in the cassette are harvested by the Parsortix PC1 system for use in subsequent downstream assays. The end user is responsible for the validation of any downstream assay. The standalone device, as indicated, does not identify, enumerate or characterize CTCs and cannot be used to make any diagnostic/prognostic claims for CTCs, including monitoring indications or as an aid in any disease management and/or treatment decisions.

2. Special conditions for use statement(s):

For Prescription Use only

For *in vitro* diagnostic (IVD) use only

3. Special instrument requirements:

The performance of the Parsortix PC1 device was assessed from blood samples collected in Becton Dickinson K₂EDTA tubes.

I. Device Description:

The Parsortix[®] PC1 system is a bench top laboratory instrument consisting of five main sub-system components:

- Parsortix PC1 instrument incorporating a computer, keypad and display, pneumatic and hydraulic components including reservoir bottles and tubes, a separation cassette mounting clamp and other electronics to control the instrument hardware and behavior.
- Parsortix PC1 Software consisting of a Windows 7 Embedded operating system together with dedicated Parsortix PC1 proprietary Windows application software (Software).
- A set of embedded and encrypted Protocol Files (Protocols) that are sequences of simple instructions, interpreted by the Software and used to control the instrument fluidic and hydraulic components and circuits. The Protocols supplied embedded within the Software enable the four core instrument processes: Clean, Prime, Separate, and Harvest.

- Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit which contains Separation Cassettes (n = 10, 50 or 100), Cleaning Cassettes [(n = 1, 5, or 10), one Cleaning Cassette for every multiple of 10 x separation cassette], Encrypted Instrument protocol file distributed on a USB memory stick as required to perform the proposed intended use, Cassette labels and one package insert (per kit) containing instructions for use and expected performance data for the Parsortix PC1 instrument, when used in conjunction with the MBC-01 Metastatic Breast Cancer Kit.
- Parsortix PC1 ICT-01 Instrument Control Test Kit which contains Control tubes containing a known, aliquoted cell suspension which is used to periodically confirm acceptable performance of the system, Separation Cassettes Polystyrene 12mL 16x100 mm tubes (n = 10 or 25) and one package insert (per kit) containing instructions for use for the ICT-01 Instrument Control Test Kit.

J. Standard/Guidance Documents Referenced:

ANGLE provided a file entitled “Parsortix PC1 System: Use of Voluntary Consensus Standards” that were conformed to in the course of their studies, these included:

Standard Designation Number	Name of Standard	FDA Recognition Number
ISO 14971:2007*	Medical devices - Applications of risk management to medical devices *Note: in the submission the standard BS EN ISO14971:2012 is referenced. This is the UK implementation of EN ISO 14971:2012. It is identical to ISO 14971:2007.	5-40
IEC 62366-1:2015	Medical devices - Part 1: Application of usability engineering to medical devices	5-114
ISO 15223-1:2016	Medical devices - Symbols to be used with medical device labels, labelling, and information to be supplied - Part 1: General requirements	5-117
ISO 62304:2006 +A1:2016	Medical device software - Software life cycle processes	13-79
EP06-A	Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach	7-193
EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition	7-266
EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition	7-251
EP07-A3	Interference Testing in Clinical Chemistry	7-275

Standard Designation Number	Name of Standard	FDA Recognition Number
EP09-A3	Measurement Procedure Comparison and Bias Estimation Using Patient Samples	7-296

A number of non-recognized Consensus Standards were also referenced in the document.

K. Test Principle:

The Parsortix PC1 device is a computer controlled programmable microfluidics and pneumatics system that can apply samples, cleaning/rinsing agents, and reagents through internal fluidic pathways and a mounted separation cassette. The system is configured and operated in accordance with specific programmable sequences of simple instructions in a proprietary format. The on-board computer also provides user interface functionality, including the operation of a simple internal user keypad and display as well as external PC monitor and keyboard connections. The external monitor and keyboard are specified for ANGLE use only. There is a dedicated user interface implemented and no user interaction with the embedded Windows operating system is required. The computer controlled programmable fluidics and pneumatics system enables precise control over the movement of fluids and air through a number of internal pathways, including through the attached single-use Parsortix GEN3 separation cassette when mounted in the reusable cassette clamp assembly.

The system contains an on-board buffer reservoir, a cleaning fluid reservoir, a priming fluid container, and a waste receptacle to capture spent fluids and non-retained blood components. Blood is progressed from the blood sample tube under controlled pressure conditions and routed through the separation cassette to enable cell separation and capture. The typical blood separation rate is approximately 5mL per hour.

Buffer, priming fluids, and cleaning fluids are drawn from the external bottles and tubes and routed through the internal fluidic components including the separation cassette. This enables:

- Priming of the system before use to remove air from the internal components and the separation cassette;
- Rinsing (with buffer) of the tubing to ensure that the entire blood sample has gone through the cassette to complete a separation with minimal sample wastage; and
- Thorough cleaning of the system after use in preparation for the next operational cycle.

An external manual harvest valve enables cells separated and captured from a blood sample to be eluted from the GEN3 separation cassette into an external vessel for further, user-defined, downstream analysis.

Through the precision control of the Parsortix® PC1 system pneumatic and fluidic circuits, syringe pump and multi-way valves, the instrument operates the four core instrument processes:

- *Prime* – the application of fluid to eliminate air bubbles from the fluidic system and to prime the system and a new separation cassette to make it ready to receive samples and/or reagents (Current time required: ~10 minutes);
- *Separate* – movement of the sample attached to the sample mount (e.g. blood from a blood sample tube) through the separation cassette to perform cell separation and capture within the cassette and rinsing (with buffer) of the tubing to ensure that the entire sample has gone through the cassette to complete a separation with minimal sample wastage (Current time required: ~2 hours, but is volume/patient dependent);
- *Harvest* – Internal instrument tubing is flushed to remove residual blood cells (“pre-harvest flush”) and then cells captured in the separation cassette are eluted into an external vessel for further analyses (Current time required: ~16 minutes);
- *Clean* – the application of cleaning fluid to remove contamination, debris and residual reagents from the system followed by buffer to rinse the cleaning fluid from the system (Current time required: ~41 minutes).

Studies to demonstrate performance of Parsortix PC1 system

In the studies presented below, Parsortix PC1 system performance was demonstrated using blood samples from both healthy volunteers and metastatic breast cancer (MBC) patients or contrived samples using cultured cells spiked into donor blood. In general, for demonstrating performance of devices used for CTC identification and enumeration, cultured cell lines have been employed. CTC cell lines are not available commercially and have only been established in an experimental setting. In addition, the scarcity and difficulty in obtaining clinically derived CTCs via patient samples in sufficient numbers and volume necessary to conduct the analytical validation studies make it difficult to demonstrate the performance of devices for CTC enrichment/isolation. For these reasons, a number of device performance studies described below were performed using contrived samples consisting of peripheral whole blood spiked with pre-determined numbers of cultured cells that were representative of the numbers and concentration of CTCs expected to be observed in the intended use population.

Considering the wide heterogeneity of breast cancer tumors and its cell types, a set of cell lines were chosen as representative of breast cancer phenotypes (SKBR3, MCF7, and Hs 578T). These three cell lines are morphologically distinct cell lines which represent three of four morphological subtypes as classified by Kenny et al (Mol. Oncol. **1**, 84–96 (2007)). CTCs isolated from breast cancer patients show high morphological intra- and inter- patient

heterogeneity (please refer to Table 1 for the cells used in the analytical studies and their unique representation of breast cancer features).

Table 1. Characteristics of Cultured Cells used in Studies compared to CTCs

Type of Cell (Cell line or patient-derived)				
	SKBR3	MCF7	Hs 578T	MBC patients derived CTCs
Breast Cancer Subtype	HER2 enriched	Luminal A	Basal/Triple Negative	All Subtypes
Phenotype	Epithelial	Epithelial	Mesenchymal	Epithelial, EMT & mesenchymal
Receptor Status	ER/PR- and HER2+	ER/PR+ and HER2-	Triple negative	ER/PR+/- and HER2+/-
Morphology	Grape-like	Mass	Stellate	Highly Polymorphic
Average Cell Size	15-17 μm^1	16.5 μm^1	No Data	• 13.1 μm^1 • 11.79 (4.51-33.11) μm^2 • 13.1 (12-25) μm^3 • 11 (6-16) μm^4

The selection of these three breast cancer cell lines was based on their differing phenotype, size, number, and morphology. All of these characteristics are features that could impact the ability of the Parsortix PC1 system to capture these cells. By use of three representative cell lines, the broad range of cell types (i.e., epithelial, mesenchymal, HER2+/HER2- status, hormone receptor (HR) status, and stellate, grape-like, and mass shapes) that could be found in the blood of MBC tumor patients corresponding to CTCs can be approximated. The use of three representative cells lines (SKBR3, MCF7, and Hs 578T cells) in the analytical studies allow for reproducibility and comparability of results and demonstrates performance of CTC isolation that is achieved by the Parsortix PC1 system.

Performance of the Parsortix PC1 device has been demonstrated using both spiked samples (as described in section L below) and intended use clinical samples (as described in section M below).

L. Performance Characteristics

¹ Coumans, F. A. W., van Dalum, G., Beck, M. & Terstappen, L. W. M. M. Filter characteristics influencing circulating tumor cell enrichment from whole blood. PloS One 8, e61770 (2013).

² Zhao, P. et al. Establishment and Characterization of a CTC Cell Line from Peripheral Blood of Breast Cancer Patient. J. Cancer 10, 6095–6104 (2019).

³ Hao, S.-J., Wan, Y., Xia, Y.-Q., Zou, X. & Zheng, S.-Y. Size-based separation methods of circulating tumor cells. Adv. Drug Deliv. Rev. 125, 3–20 (2018).

⁴ Coumans, F., van Dalum, G. & Terstappen, L. W. M. M. CTC Technologies and Tools. Cytom. Part J. Int. Soc. Anal. Cytol. 93, 1197–1201 (2018).

Performance using spiked samples: A number of studies were performed using blood samples collected from healthy donors that were spiked with predetermined numbers of cultured tumor cells as a “model system” to demonstrate the analytical performance of the Parsortix PC1 system. The results from these studies are presented in the following sections:

1. Cell Recovery Studies

This study evaluated the ability of the Parsortix PC1 system to recover high levels (between ~125 and ~1,000) of live SKBR3 cells, a cell line derived from an ER(-)/ PR(-) HER2+ metastatic pleural effusion. For ease of detection, the cells were fluorescently labeled (green) and spiked into 7.5mL of healthy volunteer (HV) whole blood. The study was performed on eight (8) PC1 instruments; separations were performed for four (4) different dilution derived spike levels (~1000, ~500, ~250, or ~125) over a period of six (6) non-consecutive days. Blood was collected from a total of 12 donors, with 4 tubes of blood collected from each of two donors on each of the 6 testing days. The harvests from the 8 instruments were collected into separate, appropriately labeled wells of a black, flat bottom 96-well plate that was covered and allowed to sit (protected from light) for 1 hour at room temperature to allow the cells to settle to the bottom of the wells. While the cells were settling, one operator counted the number of labelled SKBR3 cells remaining in each cassette using fluorescence microscopy. The best fit model for the live SKBR3 cells was determined to be the first order linear regression model, indicating that the Parsortix PC1 system was linear over the range of ~125 to ~1,000 live SKBR3 cells spiked into 7.5mL of blood. The linear model had a slope of 0.6544, indicating an average recovery rate of ~65% (95% CI = 62% - 69%) over the range of ~125 to ~1,000 live SKBR3 cells.

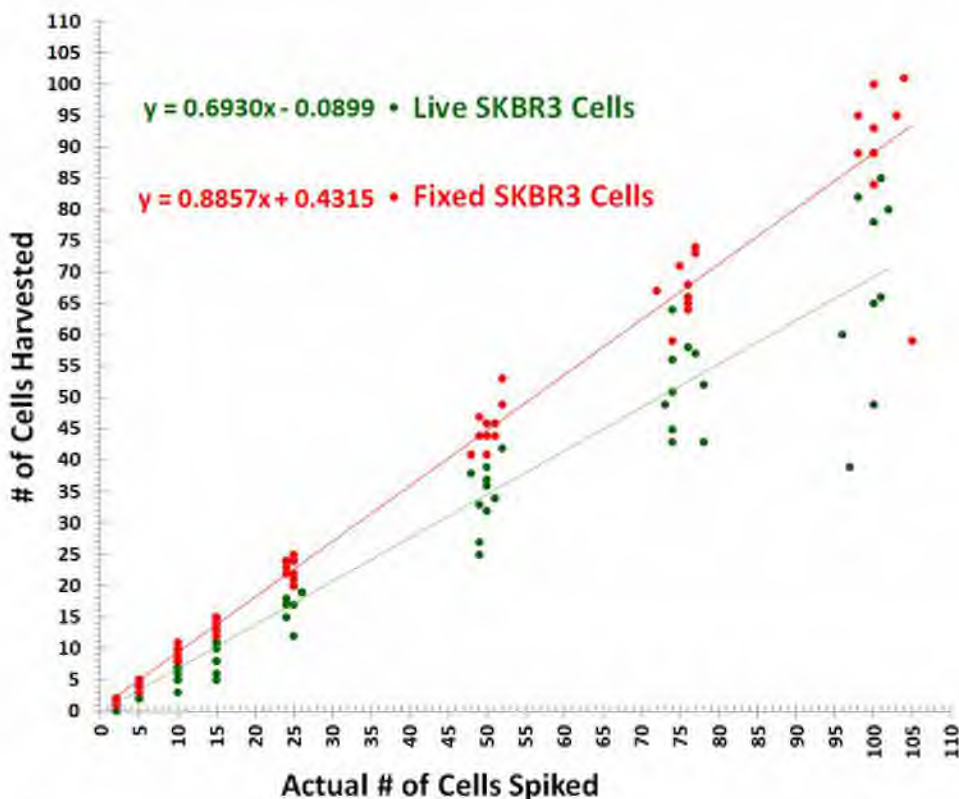
A separate study was conducted using spike levels of between 2 and ~100 live and fixed SKBR3, live MCF7, and live Hs578T cells into blood. This spiking range was chosen to represent cell levels within the range of CTCs expected to be observed in a 10mL blood sample from the majority of metastatic breast cancer patients. On each day of the study, eight (8) 10mL K₂EDTA tubes of blood were obtained from one (1) healthy female donor (Age 18-70); for a given cell line, all 10 donors were different. The study was run for 10 days, with 8 different spiking levels [2, 5, 10, 15, ~25 (24-26), ~50 (48-52), ~75 (72-78) and ~100 cells (95-105)] on eight (8) PC1 instruments. The cells were processed and counted as described above.

The best fit model for all three of the live cell lines was determined to be the first order linear regression model, indicating that the Parsortix PC1 system was linear over the range of 2 to ~100 live cells spiked into 7.5mL of blood. For SKBR3 cells, the linear model had a slope of 0.6930, indicating an average recovery rate of ~69% (95% CI = 65% - 73%) over the range of 2 to ~100 live SKBR3 cells. For MCF7 cells, the linear

model had a slope of 0.7581, indicating an average recovery rate of ~76% (95% CI = 73% - 79) over the range of 2 to ~100 live MCF7 cells. For Hs578T cells, the linear model had a slope of 0.7654, indicating an average recovery rate of ~76% (95% CI = 74% - 79%) over the range of 2 to ~100 live Hs 578T cells.

To test the efficiency and directly compare the performance of the cell harvesting of both fixed and live cells using the PC1 device, eight 7.5mL blood samples from each of 10 healthy female donors were spiked with 2, 5, 10, 15, ~25, ~50, ~75, and ~100 fixed and fluorescently labeled cultured breast cancer cells (SKBR3). These samples were then processed, and the recovered cells counted, as described above.

Figure 1. Comparison of Recovery of Live vs. Fixed SKBR3 Cells Using Parsortix PC1 System



The results (Figure 1) demonstrate that the Parsortix PC1 device captures fixed SKBR3 cells more efficiently than live cells. The Parsortix PC1 system was able to recover a significantly higher proportion of fixed SKBR3 cells spiked into 7.5mL blood (88% on average) compared to the recovery of live SKBR3 cells spiked into 7.5mL of blood (69% on average). It also shows that there is a higher degree of variability in the recovery of live, pre-labeled SKBR3 cells compared to that of fixed, pre-labeled SKBR3 cells.

The repeatability for the harvest of the live SKBR3, MCF7, and Hs 578T cells, as well as fixed SKBR3 cells, spiked into 7.5mL blood appeared to be proportional to the number of cells spiked rather than constant, with higher repeatability estimates being observed as the numbers of cells spiked increased. Conversely, the percentage difference standard deviation (SD) estimates were observed to decrease as the numbers of cells spiked increased, with pooled average % difference SD repeatability estimates of 15% to 20%.

2. Detection Limit

To evaluate the detection limit of the Parsortix PC1 System, a range of spike levels using three different breast cancer cell lines (SKBR3, Hs 578T, and MCF7) were spiked into a minimum of sixty (60) 7.5mL healthy donor blood samples for each cell line and each spike level tested. Aliquots of blood were spiked with one (1) to four (4) live, fluorescently-labelled SKBR3 or Hs 578T cells and one (1) to five (5) live, fluorescently pre-labelled MCF7 cells. The study was performed over 14 days. The detection limit is defined as the minimum number of live, fluorescently labeled tumor cells spiked into a 7.5mL blood sample required to recover at least one spiked tumor cell using the Parsortix PC1 system $\geq 95\%$ of the time. The study results showed that the minimum number of SKBR3, Hs 578T, and MCF7 cells required to be present in a 7.5mL blood sample to recover at minimum one (1) cell using the Parsortix PC1 system were 3, 4 and 5 cells, respectively. In a separate part of the previous study, one or more separate 7.5mL aliquots of blood from 63 different self-declared healthy donors were left unspiked for assessment of the limit of blank, which was assumed to be 0 cells per 7.5mL of blood. Based on these results, the limit of blank for the Parsortix PC1 system was determined to be 0 cells.

3. Blood Volume

Seven and one half (7.5) mL is the desired volume of blood for processing on the Parsortix PC1 system. The main objective of this study was to assess whether different volumes of blood have an impact on the device performance. The study also compares the recovery rates of direct harvesting of cells using the Parsortix PC1 device from different volumes of blood and compared to the use of Cytospin™ slides for the deposition of the harvested cells (compared to harvesting directly into a 96-well plate).

Table 2. Summary of Percentage Harvested Results for Blood Volume testing (Samples where cells were harvested directly into wells on a 96-well plate)

	%SKBR3 Cells Harvested into 96-well Plates		
Parsortix PC1 System Blood Volume Study using live SKBR3 cells spiked in HV Blood	7.5mL 96-well Plate Harvests	5mL 96-well Plate Harvests	10mL 96-well Plate Harvests
Mean	71.1%	62.3%	66.3%
StDev	14.1%	15.8%	16.5%
Variance	2.0%	2.5%	2.7%
Avg Difference from 7.5mL		-8.8%	-4.8%
95% CI from Avg. Difference		(-22.0% to 4.4%)	(-18.4% to 8.8%)

Table 3. Summary of Percentage Harvested Results for Blood Volume testing (Samples where harvested cells were deposited onto Cytospin™ slides)

	% of Harvested SKBR3 Cells Deposited onto Cytospin™ Slides		
Parsortix PC1 System Blood Volume Study using live SKBR3 cells spiked in HV Blood	7.5mL Cytospin Slide Deposits	5mL Cytospin Slide Deposits	10mL Cytospin Slide Deposits
Mean	23.5%	24.2%	29.1%
StDev	16.3%	20.0%	15.2%
Variance	2.6%	4.0%	2.3%
Avg Difference from 7.5mL		0.7%	5.6%
95% CI from Avg. Difference		(-15.9% to 17.3%)	(-8.3% to 19.5%)

Results from these three blood sample sizes (Tables 2 and 3) show that blood volume has no impact on the efficiency of the Parsortix PC1 system to capture and harvest target cells. The results also demonstrate that depositing cells harvested by the Parsortix PC1 system onto Cytospin™ slides using cytocentrifugation results in the loss of a significant number of both SKBR3 cells (Table 3) and residual nucleated cells. This observation (the cell loss due to the cytocentrifugation process) needs to be taken into consideration if additional analysis is intended with the cells derived from cell harvests.

4. Blood Stability

The main objective of this blood stability study was to assess and quantify the impact of storage conditions (i.e., temperature and time) on the performance of the Parsortix PC1 system using blood samples from healthy donors (spiked with live, cultured, pre-labeled SKBR3 cells) that were stored at either 4°C or room temperature (RT) for up to 72 hours following the blood draw. In the results presented in the following table, the stored samples were stored at RT or refrigerated (4°C) and processed at 24 hours (1 day after collection), 48 hours (2 days after collection) or 72 hours (3 days after collection).

Table 4. Summary of Percent Harvest Results for Blood Stability Testing

	% Harvest						
Blood Stability Testing Results	Control (0-8h, RT)	24h at RT	48h at RT	72h at RT	24h at 4° C	48h at 4° C	72h at 4° C
Mean	71.2%	81.6%	74.9%	71.1%	72.9%	72.5%	74.1%
StDev	19.0%	8.0%	11.3%	14.9%	12.5%	11.4%	11.2%
Variance	3.6%	0.6%	1.3%	2.2%	1.6%	1.3%	1.2%
Avg Difference from 7.5mL		10.4%	3.7%	-0.1%	1.7%	1.3%	2.9%
95% CI from Avg. Difference		(-0.1% to 20.9%)	(-7.8% to 15.2%)	(-13.0% to 12.8%)	(-10.3% to 13.7%)	(-10.3% to 12.9%)	(-8.6% to 14.4%)

SKBR3 cells were spiked into blood samples collected into K₂EDTA tubes. The samples were then stored at room temperature (RT) or 4°C for 24, 48, or 72 hours (harvests from these samples were compared to control samples stored at RT for 0-8 hours). The results (Table 4) showed that the storage of blood samples at room temperature or refrigerated (4°C) for up to ~72 hours after the time of collection prior to processing will have no significant impact on the ability of the Parsortix PC1 system to capture and harvest live SKBR3 cells. Additional conclusions drawn from the study: 1) Storage of blood samples at room temperature for more than 4 hours (or samples refrigerated (4°C) for more than ~48 hours) after the time of collection prior to processing will result in the capture and harvest of significantly higher numbers of residual nucleated cells by the Parsortix PC1 system. 2) Storage of blood samples at room temperature for more than ~24 hours after the time of collection prior to processing on the Parsortix PC1 system may result in significantly longer processing times; however, storage of blood samples refrigerated (4°C) for up to ~72 hours after the time of collection prior to processing will not significantly impact sample processing time.

5. Cell Carryover

Two 7.5mL blood samples spiked with a high number (~1,000) of fluorescently labeled, live SKBR3, Hs 578T, and MCF7 cells were processed on the Parsortix PC1 system followed by five 7.5mL Phosphate-buffered saline (PBS) samples which were processed on the same systems. This process was repeated eleven (11) times on four (4) different Parsortix PC1 system. Parsortix PC1 harvests from the PBS samples were examined for the presence of any pre-labeled spiked cells or other nucleated cells carried over within the instrument from the previous blood sample runs. Of the 220 PBS harvests, none showed any fluorescently labeled cells being carried over, suggesting that the established cleaning procedure of the Parsortix PC1 system between sample runs ensures the absence of any cell carryover between samples.

6. Cleaning reagent carryover

A series of experiments were performed to determine if residual cleaning reagent, used to clean the Parsortix PC1 device between processing runs, can interfere with subsequent cell recovery. Samples of 10mL of deionized water were processed on the Parsortix PC1 systems over four (4) days immediately after completing the specified cleaning process for the system using a 10% solution of ProKlenz® 120 cleaning detergent. Two (2) of these deionized water samples were processed on each day using ten (10) Parsortix PC1 systems where the standard running buffer (1x PBS) was replaced with deionized water. The specified concentration of cleaning reagent (10%) was tested at 2 and 3 times this level (e.g. 20% and 30% solutions of ProKlenz® 120). The waste solutions and harvests produced following the processing of each of the water samples were tested for traces of ProKlenz 120 by measuring both the pH of the waste solutions and resulting harvests and the potassium content of the waste solutions and the resulting harvests (pH and potassium are both measurable components of ProKlenz 120). The use of a 10% solution of the cleaning reagent resulted in no more in 0.01% residual cleaning detergent, a level demonstrated not to have any impact on the recovery of target cells, their morphology, and the molecular evaluation of the RNA isolated from the captured cells.

7. Cassette Lot Study

The purpose of these studies was to determine the average percentage of harvested green fluorescently labeled, fixed SKBR3 cells (~20 green fluorescently labelled fixed SKBR3 cells) spiked into PBS from a Parsortix Control (PC) tube using multiple lots of Parsortix GEN3 Cell Separation Cassettes on a minimum of 20 Parsortix PC1 instruments. This information was also used to establish the upper and lower acceptance criteria for cassette performance.

Each one of the fifteen (15) cassette lots were evaluated on all operable Parsortix PC1 systems located at the ANGLE laboratory (a minimum of 20 instruments) and the percentage of green, fluorescently labeled cells contained in the Parsortix Control Tube (PCT-001) spiked into 2.5mL of PBS and processed by the Parsortix PC1 systems that are captured and harvested, was determined. For each individual sample, the number of green cells in the harvest was determined by two (2) independent readers, while the number of green cells remaining in the cassettes after harvest was determined by a single reader. A total of 328 runs were used to perform the study. These values, along with the number of cells that were contained within each of the Parsortix Control Tubes, were used to calculate the % harvest (percentage of cells harvested) and % capture (percentage of cells harvested plus cells remaining in the cassettes after harvest) results for each operable Parsortix PC1 instrument for each cassette lot. The overall mean % harvest was 81.4%, with a standard deviation (SD) of 14.4% and a %CV of 17.7%. The lower and

upper limits for the % harvest were 52.6% and 100%, respectively. The overall mean % capture was 84.0%, with a SD of 13.2% and a %CV of 15.6%. The lower and upper limits for the % capture were 57.6% and 100%, respectively. These values were used as the acceptance ranges for the testing of cassettes.

8. Interfering Substances

Exogenous substances: Fluorescently labeled, cultured live breast cancer cells (SKBR3) spiked into blood samples were subjected to cancer drugs that could interfere and recovery of the cells by the Parsortix PC1 system was compared to untreated controls. A maximum single dose of each of the following cancer drugs was tested: Tamoxifen Citrate, Acetaminophen, Mitomycin C[®], Paclitaxel, Rosuvastatin Calcium, Cisplatin, Alendronate Sodium, 5-Fluorouracil, Doxorubicin Hydrochloride, and Dexamethasone. No significant differences in the number of captured or harvested SKBR3 cells were detected, indicating that these drugs, when used at the concentrations tested in the study, do not interfere with the ability of the Parsortix PC1 system to capture and harvest target cells. The presence of ~80µg/mL of Paclitaxel in blood was found to potentially have an impact on the functioning of the Parsortix system and may cause the occasional loss of samples and/or reduction in the quality of the harvest. In addition, and consistent with the results presented above, the presence of ProKlenz 120 at a concentration up to 0.1% (determined in the study above) does not negatively impact the recovery rate of target cells or the sample's processing time.

Endogenous substances: High levels of albumin or triglycerides did not have an impact on the number of harvested cells or the sample processing time when compared to control samples. Different hematocrit levels did not interfere with the ability of the Parsortix PC1 system to capture and harvest target cells. Higher hematocrit levels, however, increased the sample processing time and moderately increased the number of residual white blood cells in the harvest while lower hematocrit levels significantly increased the average number of nucleated blood cells in the harvest.

White Blood Cells (WBCs): A high white blood cell (WBC) count (up to an average of 16×10^9 cells/L) was not found to interfere with the efficiency of the Parsortix PC1 system to capture and harvest SKBR3 cells. However, elevated WBC levels lead to a significant increase in the average number of nucleated blood cells harvested by the Parsortix PC1 system, which could lead to interference when using the population of cells harvested (including CTCs if present in sufficient numbers in the blood sample) in any downstream applications. To demonstrate that the levels of background cells (i.e. primarily WBCs) in Parsortix harvests did not negatively impact the downstream processing of the harvested cells, two additional studies were conducted.

The first study was designed to demonstrate that increased levels of WBCs did not interfere with a representative molecular analysis test (the qPCR assay used in the ANG-002 study as described in Section M below). To assess this, known numbers of cultured tumor cells were mixed with one of three (3) different levels of spiked WBCs (0, ~5,000 and ~40,000 cells). The level of ~5,000 cells represents the approximate average number of WBCs observed in the Parsortix harvests obtained from the control samples processed in the interference assessments. In brief, 0 cells (Negative Controls), 1 SKBR3 + ~20 Hs 578T cells (Low CTC samples), or ~10 SKBR3 + ~50 Hs 578T cells (High CTC samples) were combined with WBC cells (spiked cells) at levels of 0, ~5,000 and ~40,000. Both SKBR3 and Hs 578T were included in the contrived samples so that detection of both the epithelial and mesenchymal cancer-related target genes could be demonstrated with the qPCR assay in the presence of the background cells. The genes tested were the following: GAPDH and B2M (housekeeping genes), GYPA (a nucleated red blood cell marker), PTPRC (a white blood cell marker), EPCAM and KRT19 (epithelial cell markers), ERBB2 (also known as HER2, is a breast tumor marker), and TWIST and SNAI2 (mesenchymal cell markers). The samples containing WBCs only, with no spiked cultured tumor cells, were used as background controls to demonstrate the specificity of the ANG-002 qPCR assay in the presence of WBCs at the average level expected to be found in Parsortix harvest (~5,000 WBCs) as well as at a much higher level not expected to be seen in a typical Parsortix harvest that could introduce interference (~40,000 WBCs). The samples tested also included positive control samples which were contrived harvests containing only the spiked cultured tumor cells and no WBCs.

When using either a set Ct (threshold cycle) threshold of <35.0 or gene-specific Ct thresholds to define positivity, there were no differences in the proportions of lysates with positive expression for any of the genes (or combinations of genes) between the two different WBC Background groups within the Low CTC samples (Control and Test) or within the High CTC samples (Control and Test) (this excluded the ERBB2 gene, due to the observed background expression of this gene in WBCs as ERBB2 is known to be expressed in white blood cells). These results showed that an excessive number of WBCs did not negatively impact the performance of the qPCR assay for all selected genes except for ERBB2. The results of this study demonstrate the importance of carefully selecting markers that can be differentially detected in CTCs in the presence of the expected WBC background and carefully optimizing and characterizing the downstream assay to work with this level of purity of the target cell population.

The second study was designed to demonstrate that the levels WBCs in Parsortix harvests did not negatively impact the cytology, fluorescence in situ hybridization (FISH) or immunofluorescence (IF) evaluation methods that were performed in the Clinical Studies

(described in Section M below). The contrived harvests used for these assessments consisted of a single cultured tumor cell level of ~10 live SKBR3 cells (range of 9-11 cells) combined with WBCs spiked at levels of 0, ~5,000 and ~40,000 cells. The cells in the contrived harvests were deposited onto positively charged glass Cytospin slides using a cytocentrifugation method, and the slides were then prepared for the specific testing required. The following three tables (Tables 5, 6, and 7) show the results from these representative studies:

Table 5. Summary of the percentage of SKBR3 cells observed on the Kwik-Diff stained cytology slides generated from the Positive Controls, Average WBC Test Samples, and the Excessive WBC Test Samples.

	% of SKBR3 Cells Observed on <i>Kwik-Diff</i> Stained Cytology Slides		
WBC Background Interference Studies using live SKBR3 Cells	Positive Controls	Average WBC Test Samples	Excessive WBC Test Samples
Mean	7.4%	11.0%	15.1%
StDev	13.7%	11.6%	14.0%
Variance	1.9%	1.4%	2.0%
Avg Difference from Pos Control		3.6%	7.7%
95% CI from Avg. Difference		(-7.2% to 14.4%)	(-4.1% to 19.5%)

Table 6. Summary of the percentage of SKBR3 cells observed on the IF-stained cytology slides generated from the Positive Controls, Average WBC Test Samples, and the Excessive WBC Test Samples.

	% of SKBR3 Cells Observed on IF-Stained Cytology Slides		
WBC Background Interference Studies using live SKBR3 Cells	Positive Controls	Average WBC Test Samples	Excessive WBC Test Samples
Mean	27.8%	24.2%	32.9%
StDev	23.4%	17.7%	24.4%
Variance	5.5%	3.1%	5.9%
Avg Difference from Pos Control		-3.6%	5.1%
95% CI from Avg. Difference		(-21.2% to 14.0%)	(-15.2% to 25.4%)

Table 7. Summary of the percentage of SKBR3 cells observed on the FISH-stained cytology slides generated from the Positive Controls, Average WBC Test Samples, and the Excessive WBC Test Samples.

	% of SKBR3 Cells Observed on FISH- Stained Cytology Slides		
WBC Background Interference Studies using live SKBR3 Cells	Positive Controls	Average WBC Test Samples	Excessive WBC Test Samples
Mean	17.4%	28.8%	22.1%
StDev	24.8%	16.8%	16.5%
Variance	6.1%	2.8%	2.7%
Avg Difference from Pos Control		11.4%	4.7%
95% CI from Avg. Difference		(-6.6% to 29.4%)	(-13.2% to 22.6%)

These results show that an excessive number (~40,000) of WBCs did not have a significant impact on the quality of the WBCs or SKBR3 cells observed on the Kwik-Diff stained, IF-stained or FISH-stained cytology slides. Similarly, the results obtained for the qPCR evaluation showed that an excessive number of WBCs did not have a negative impact on the overall performance of the qPCR assay, the exception being ERBB2, as noted above.

Therefore, WBCs are not considered to be an interferent in any of the following downstream analyses (cytology, immunofluorescence staining, FISH, and qPCR) that were evaluated using logical representative targets. These downstream analyses were not performed for any downstream testing claims, rather, end users must validate use with any subsequent tests and collection devices. In conclusion, the number of residual background cells present in the Parsortix harvests study samples is not believed to have prohibited the downstream analyses presented in the clinical study reports from being successfully conducted.

9. Reproducibility and Repeatability

A number of studies were performed to assess the precision of the Parsortix PC1 system. Several of them used cells lines (live or fixed cells) spiked into aliquots of PBS while others employed live cells spiked into blood. These studies included:

Ten-day precision study: Approximately 20 fixed and fluorescently labeled, breast cancer cells (SKBR3) suspended in 1% bovine serum albumin (BSA), 2mM ethylene diamine tetra-acetic acid (EDTA) PBS solution were spiked into 2.5mL aliquots of PBS and used to assess the reproducibility of the Parsortix PC1 system using three (3) different lots of Parsortix GEN3 Cell Separation Cassettes (6.5 µm critical gap). The spiked PBS samples were separated using ten (10) Parsortix PC1 instruments during two (2) runs per instrument per day over ten (10) days for each cassette lot tested, generating a total of 200 measurements per cassette lot. The imprecision estimates for the three (3) Cassette Lot (C, F and G) 10-day precision studies using fixed, pre-labeled SKBR3 cells spiked

into 2.5mL of PBS achieved % coefficients of variation (%CVs) within acceptable limits. The overall average harvest percentages for the different cassettes ranged from 80.7% to 82.2%, the repeatability %CV estimates ranged from 12.9% to 15.9%, and the within-laboratory %CV estimates ranged from 13.4% to 15.9%. The combined cassette lot repeatability %CV estimate for the Parsortix PC1 system was 14.4%, with a reproducibility %CV estimate of 14.5%.

Table 8. 10-day single site 3-lot precision study by spiking fixed SKBR3 cells into PBS

Cell Line	Spiked cell counts	N	Mean % Harvest	Within-run (Repeatability)		Between Run		Between Day		Between Lot		Within laboratory	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Fixed SKBR3	~20	600	81.3%	11.7%	14.4%	0.0%	0.0%	1.3%	1.6%	0.0%	0.0%	11.8%	14.5%

Twenty-day reproducibility: Approximately 20 fixed and fluorescently labeled breast cancer cells (SKBR3) suspended in 1% BSA, 2mM EDTA PBS solution were spiked into 2.5mL aliquots of PBS and used to assess the reproducibility of the Parsortix PC1 system across three (3) different sites. The spiked PBS samples were separated using twenty (20) instruments in total across the three (3) sites during two (2) runs per instrument per day over a total of twenty (20) days, generating a total of 800 data points. The overall average harvest percentages at the individual sites ranged from 65.4% to 81.6% with an overall mean of 75.3%, the repeatability %CV estimates ranged from 14.0% to 22.9%, and the within-laboratory %CV estimates ranged from 14.2% to 23.4%. The multisite repeatability %CV estimate for the Parsortix PC1 system was 17.0%, with a reproducibility %CV estimate of 20.6%.

Table 9. 20-day 3-site reproducibility study by spiking fixed SKBR3 cells into PBS

Cell Line	Spiked cell counts	N	Mean % Harvest	Within-run (Repeatability)		Between Run		Between Day		Between Lot		Within laboratory	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Fixed SKBR3	~20	800	75.3%	12.8%	17.0%	2.5%	3.3%	0.0%	0.0%	8.4%	11.2%	15.5%	20.6%

Single site precision: Blood samples were drawn from two healthy donors each day and 7.5mL aliquots were spiked with approximately twenty (20) live and fluorescently labeled cultured breast cancer cells (SKBR3). The spiked blood samples were processed using the Parsortix PC1 system and the number of fluorescently labeled cells in each harvest were counted using a fluorescence microscope and the percentage of cells harvested determined. The study was conducted over twenty (20) non-consecutive days using ten (10) instruments with two (2) runs per instrument per day (morning and

afternoon) by two (2) operators (the operators alternated between the morning and afternoon runs). The imprecision estimates from this 20-day precision study using live, fluorescently-labeled SKBR3 cells spiked into 7.5mL of blood achieved % CVs within acceptable limits. The overall average % harvest was 70.4%, the repeatability %CV was 21.1%, and the within-laboratory %CV was 22.0%.

A similar study to the one detailed above was carried out using fixed (instead of live), fluorescently labeled cultured breast cancer cells (SKBR3). The overall average harvest percent for the fixed SKBR3 cells was 89.4%, the repeatability %CV was 10.2%, and the within-laboratory %CV was 10.3%.

The repeatability %CV estimate from the combined 20-day precision studies (fixed and live SKBR3 cells spiked into blood) for the Parsortix PC1 system was 15.4%, with a reproducibility %CV estimate of 23.2%.

Table 10. 20-day single site precision study by spiking fixed and live SKBR3 cells into blood

Cell Line	Spiked cell counts	N	Mean %Harvest	Within-run (Repeatability)		Between Run		Between Day		Within Laboratory	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Fixed SKBR3	~20	400	89.4%	9.2%	10.2%	1.1%	1.2%	0.2%	0.2%	9.2%	10.3%
Live SKBR3	~20	400	70.4%	14.8%	21.1%	4.3%	6.2%	0.0%	0.0%	15.5%	22.0%

5-day single site precision: Additional single site 5-day precision studies were also performed. These were performed using a single lot of cassettes for each of the three (3) cell lines at three (3) different live cell spiking levels. For these additional precision studies, contrived precision samples consisting of 7.5mL aliquots of blood drawn from healthy women that were spiked with a known number (i.e., 5, 10, or ~50) of live cells using one of the pre-labeled cell lines (SKBR3, MCF7, or Hs 578T) were used. For the ~50 cell spike level, the actual number of pre-labeled cells spiked into each sample was accurately determined using fluorescence microscopy and was between 48 to 52 cells. For each cell line, the results evaluated in the precision analyses were the percentage of cells harvested from each contrived sample (i.e., the number of cells observed in the harvest of each sample divided by the actual number of cells spiked into each sample). For each cell line at each spike level, a set of ten (10) Parsortix PC1 instruments were used, with two (2) runs being conducted on each instrument each day over a period of five (5) non-consecutive days. This resulted in 10 measurements per instrument and a total of 100 measurements for each cell line spike level (5 days x 2 runs per day x 10 instruments/run). The entire set of 5-day precision studies resulted in a total of 900

measurements (300 measurements for each cell line, 100 measurements per spike level). The results from the study are presented in Table 11 below.

Table 11. 5-day single site within-laboratory precision study

Cell Line	Spiked cell counts description	N	Mean % Harvest	Within-run (Repeatability)		Between Run		Between Day		Within Laboratory	
				SD	CV%	SD	%CV	SD	%CV	SD	%CV
Live SKBR3	5	100	73.6%	22.8%	31.0%	6.7%	9.1%	0.0%	0.0%	23.7%	32.2%
	10	100	70.4%	18.5%	26.3%	0.0%	0.0%	4.4%	6.3%	19.1%	27.1%
	~50 (48-52)	100	72.1%	13.8%	19.1%	0.0%	0.0%	0.0%	0.0%	13.8%	19.1%
Live MCF7	5	100	68.0%	22.0%	32.4%	7.3%	10.7%	1.3%	1.9%	23.2%	34.1%
	10	100	68.4%	18.4%	26.9%	0.0%	0.0%	6.3%	9.2%	19.5%	28.5%
	~50 (48-52)	100	76.2%	11.3%	14.8%	0.0%	0.0%	3.4%	4.5%	11.8%	15.5%
Live Hs 578T	5	100	66.6%	19.9%	29.9%	5.9%	8.9%	3.6%	5.4%	21.0%	31.5%
	10	100	63.5%	17.8%	28.0%	0.0%	0.0%	7.5%	11.8%	19.3%	30.4%
	~50 (48-52)	100	70.0%	8.6%	12.3%	0.0%	0.0%	3.4%	4.9%	9.3%	13.3%

The imprecision estimates from the individual 5-day precision studies using five (5), ten (10) and approximately fifty (~50) live SKBR3, MCF7 or Hs 578T cells spiked into 7.5mL of blood are presented in Table 11. The overall average harvest percentages for each of the cell lines/spike levels ranged from 63.5% to 76.2%, the within-run repeatability %CV estimates ranged from 12.3% to 32.4%, and the within laboratory %CV estimates ranged from 13.3% to 34.1%.

The repeatability and reproducibility %CV estimates for all of the SKBR3, MCF7, and Hs 578T precision samples processed on Parsortix PC1 systems combined (spike levels between 5 - ~50 cells) were both 26.3%. An additional observation from the study was that the repeatability and reproducibility imprecision estimates decreased as the number of spiked cells in the sample increased. The results illustrate the variability of the input cell number compounded by the device recovery, particularly at the lower cell number input. Perhaps this is not unexpected, since the gain or loss of a single cell at the 5-cell sample level represents a 20% change in recovery.

M. Evaluation of the Parsortix® PC1 system with clinical samples

ANGLE Europe Limited provided data from two studies to support the clinical performance of the Parsortix PC1 device. The first study (ANG-008) was to show that the Parsortix® PC1 system could reproducibly capture and harvest fixed, pre-labeled SKBR3 cells spiked into the peripheral blood of HV subjects and MBC patients. This study also assessed the enrichment of patient derived CTCs from a separate peripheral blood sample drawn the same HV and MBC subjects. The primary objective of the second study (ANG-002) was to demonstrate that the system can capture and harvest CTCs from the peripheral blood of MBC

patients and that the harvested cells could be used in subsequent evaluations. Details of each of the studies are provided below:

Study #1 (ANG-008)

In this study, approximately 30mL of blood was collected into 3 tubes (one 3mL K₂EDTA tube followed by two 10mL K₂EDTA tubes followed by one 7.5mL SST tube) from each HV subject and MBC patient. A total of 76 HV subjects (all of which were evaluable for one or more of the study endpoints) and 85 MBC patients (76 which were evaluable for one or more of the study endpoints). Using the 3mL K₂EDTA and 7.5mL SST tubes, a full blood workup was performed for each subject to determine whether components of the blood matrix would impact the performance of the Parsortix® PC1 system. There were no visibly significant correlations observed between any of the CBC with Differential or serum chemistry tests and the percentage of SKBR3 cells harvested from the spiked samples or with the number of CTCs observed on the IF stained cytology slides or on the Wright-Giemsa stained IF cytology slides. The only exception was the erythrocyte sedimentation rate (ESR); as the ESR increased, the amount of time required to process the blood sample concomitantly increased.

Primary Evaluation: The 10mL K₂EDTA tube with the largest volume of blood (≥ 5.0 mL of blood required for processing) was spiked with ~20 fixed, labeled SKBR3 cells using pre-prepared and precisely aliquoted spiking cell suspensions. The spiked samples were processed on Parsortix® PC1 systems using Parsortix GEN3 Cell Separation Cassettes, and the captured cells were harvested directly onto glass microscope slides. The number of pre-labeled SKBR3 cells harvested directly onto each slide was determined using fluorescent microscopy and the results recorded. A total of 76 HV subjects and 74 MBC patients had evaluable results for this primary evaluation.

Secondary Evaluation: Using the blood drawn into the remaining 10mL K₂EDTA tube (≥ 5.0 mL of blood required for processing), the ability of the Parsortix® PC1 system to capture and harvest CTCs (as identified by an IF staining method) from the blood of HV subjects and MBC patients was assessed. The proportions of HV subjects and MBC patients with one (1) or more CTCs identified using IF [CTCs defined as cells that were 4',6-diamidino-2-phenylindole (DAPI) positive, CD45 marker negative, and EpCAM and/or cytokeratin (CK) positive] on cytology slides (prepared using the same cytocentrifugation method used in the analytical studies) were determined and compared. These markers are commonly used to distinguish CTCs from other blood-derived cells. A total of 72 HV subjects and 75 MBC patients had evaluable results for this secondary evaluation.

The overall average percentage of spiked SKBR3 cells harvested directly onto slides from the 76 evaluable HV subjects and 74 evaluable MBC patients combined was 70.0% $\pm$ 15.4% (Wilson 95% CI = 62.3% to 76.7%, median = 70.7%). The average percentage of spiked SKBR3 cells harvested in the 76 evaluable HV subjects was 72.1% $\pm$ 16.1% (Wilson 95% CI = 61.1% to 80.9%, median = 75.0%) compared to 67.9% $\pm$ 14.3% (Wilson 95% CI = 56.7% to 77.4%, median = 68.4%) in the 74 evaluable MBC patients (t-test p-value = 0.0981).

The cells harvested from the blood samples processed for the IF evaluation were deposited onto charged glass cytology slides (Cytospin™ slides). Following fixation, the slides were stained using antibodies for cytokeratins 8, 18, and 19, used to identify epithelial cells and conjugated with Alexa Fluor 488, EpCAM, an epithelial cell marker conjugated with Alexa Fluor 555, and the CD markers CD45, CD11b, CD16, and CD61, markers of blood cells conjugated with APC. DAPI was used to identify the cell nucleus. In total, four (4) different channels on a Leica LAS X fluorescence microscope were used to evaluate the IF-stained slides.

Using this IF evaluation method, in the 72 HV subjects with evaluable IF results, 67 (93.1%, Wilson 95% CI = 84.9% – 97.1%) had no cells classified as being CTCs, whereas 5 (6.9%, Wilson 95% CI = 3.5% – 15.2%) had one or more cells observed on their IF slides that were DAPI+, EpCAM+ and/or CK+, and CD-. In the 75 MBC patients with evaluable IF results, 41 (54.7%, Wilson 95% CI = 43.5% – 65.6%) had no cells classified as being CTCs, whereas 34 (45.3%, Wilson 95% CI = 34.5% – 56.6%) had one or more cells observed on their IF slides that were DAPI+, EpCAM+ and/or CK+, and CD-. Similar proportions of the HV subjects and MBC patients had one or more cells classified as naked nuclei identified on their IF slides (65.3% vs. 69.3%, respectively, Fisher's exact p-value = 0.725).

The results show that a significantly larger proportion of MBC patients were found to have one or more cells observed on their IF stained cytology slides classified as CTCs compared to the HV subjects, and that equal proportions of HV subjects and MBC patients had one or more cells observed on their IF stained cytology slides that were classified as naked nuclei. A follow up study was performed to determine if the IF stained slides containing the cells harvested by the Parsortix® PC1 system could be re-stained using Wright-Giemsa reagents and evaluated by a pathologist to determine the proportion of MBC patients and HV subjects having evaluable CTCs. The proportions of cells that were classified as malignant were consistent with the number of IF stained cells presented above, indicating that the cells that were originally assessed by IF were still capable of undergoing Wright-Giemsa staining.

Study #2 (ANG-002)

The purpose of the ANG-002 study was to demonstrate that the Parsortix® PC1 device enables the capture and harvest of CTCs from the peripheral blood of patients with MBC. The primary objectives were to determine the proportion of MBC patients that had one or more observable CTCs (as determined by a qualified pathologist using cytological evaluation) harvested from their peripheral blood and that the harvested cells can be used in subsequent evaluations. The second objective was shown using several different downstream evaluations designed to detect and/or characterize CTCs in the population of cells harvested by the device. Blood samples from approximately 200 MBC patients and 200 HV subjects were collected and processed with the Parsortix® PC1 device.

Cytological Evaluation: For this evaluation, the cells harvested from a minimum of 5mL of peripheral blood were deposited onto cytology slides using the same cytocentrifugation method used in the analytical studies, fixed, and Wright-Giemsa stained using an automated stainer. Samples with no or inadequate staining of internal controls (i.e., RBCs and WBCs) were considered non-evaluable due to internal control failure. For all evaluable samples [202 from MBC patients and 192 from HVs], the total number of observable CTCs, as well as the numbers of other cells observed in each sample (i.e., atypical cells, cells of unknown origin, and naked nuclei) were determined, plotted, and summarized separately as well as aggregately for the MBC patients and HVs. The proportion (%) of MBC patients and HVs with atypical cells, benign cells of unknown origin, naked nuclei, and CTCs, alone as well as in combination, was determined using the evaluable results and compared using Fisher's exact testing. The actual numbers of each cell type in each harvest from the cytological evaluations were also summarized and plotted using the evaluable results. The proportion of subjects with epithelial-mesenchymal transition (EMT)-like features and/or CTC clusters present in the MBC patients and/or HVs where CTCs were observed was also determined.

Using this cytological evaluation method, 15.8% of the MBC patients had one or more observable CTCs as compared to 1.6% of the healthy volunteers (Table 12, below). Similarly, the percentage of MBC patients with cells classified as CTCs, atypical cells, cells of unknown origin, and/or naked nuclei observed (45.0%) was higher than that observed in the healthy volunteers (18.2%) (Table 13). The tables also depict the observed percentage breakdowns at higher numbers of CTCs observed (≥ 2 , ≥ 3 , ≥ 4 , ≥ 5 and ≥ 10) for each subject population. The information in these tables was not intended to convey cell enumeration but provides a comparison of the percentages of CTCs identified between the HV subject and MBC patient populations. Importantly, the analysis does provide some insight into the proportion of the evaluable MBC patients with higher numbers of CTCs. While 32 of 202 subjects (15.8%) had ≥ 1 CTC, only 8 of 202 (4%) had ≥ 10 CTC (or approximately 25% of those found to have CTCs). Although this is a relatively small sample size, this information should be taken into consideration when downstream applications are planned.

Table 12. Proportions of Eligible HV Subjects and MBC Patients with Adequate Wright-Giemsa Stained Cytology Slides where one or more cells were Classified as Malignant CTCs (%) [95% CI]

# CTCs Observed	Proportions of Subjects with CTCs observed in Harvests Deposited onto Wright-Giemsa Stained Cytology Slides					
	Evaluable HV Subjects	Evaluable MBC Subjects	Fisher's Exact p-value	Newly Diagnosed MBC Subjects	Recurring/Progressing MBC Subjects	Fisher's Exact p-value
0 CTC	189 (98.4%) [95.5 - 99.5%]	170 (84.2%) [78.5 - 88.5%]	---	68 (93.2%) [85.0 - 97.0%]	102 (79.1%) [71.3 - 85.2%]	---
≥ 1 CTC	3 (1.6%) [0.5 - 4.5%]	32 (15.8%) [11.5 - 21.5%]	0.0000	5 (6.8%) [3.0 - 15.0%]	27 (20.9%) [14.8 - 28.7%]	0.009
≥ 2 CTC	1 (0.5%) [0.1 - 2.9%]	29 (14.4%) [10.2 - 19.9%]	0.0000	5 (6.8%) [3.0 - 15.0%]	24 (18.6%) [12.8 - 26.2%]	0.022
≥ 3 CTC	1 (0.5%) [0.1 - 2.9%]	24 (11.9%) [8.1 - 17.1%]	0.0000	5 (6.8%) [3.0 - 15.0%]	19 (14.7%) [9.6 - 21.9%]	0.116
≥ 4 CTC	0 (0.0%) [0.0 - 2.0%]	18 (8.9%) [5.7 - 13.6%]	0.0000	4 (5.5%) [2.1 - 13.3%]	14 (10.9%) [6.6 - 17.4%]	0.304
≥ 5 CTC	0 (0.0%) [0.0 - 2.0%]	18 (8.9%) [5.7 - 13.6%]	0.0000	4 (5.5%) [2.1 - 13.3%]	14 (10.9%) [6.6 - 17.4%]	0.304
≥ 10 CTC	0 (0.0%) [0.0 - 2.0%]	8 (4.0%) [2.0 - 7.6%]	0.0007	2 (2.7%) [0.8 - 9.4%]	6 (4.7%) [2.1 - 9.8%]	0.713
TOTAL N	192	202		73	129	

Table 13. Proportions of Eligible HV Subjects and MBC Patients with Varying Numbers of Cells Classified as CTCs, Atypical Cells, Cell of Unknown Origin and/or Naked Nuclei Observed on their Wright-Giemsa Stained Cytology Slides (%) [95% CI]

# CTCs Observed	Proportions of Subjects with Non-Normal Cells Observed in Harvests Deposited onto Wright-Giemsa Stained Cytology Slides					
	Evaluable HV Subjects	Evaluable MBC Subjects	Fisher's Exact p-value	Newly Diagnosed MBC Subjects	Recurring/Progressing MBC Subjects	Fisher's Exact p-value
0 CTC	157 (81.8%) [75.5 - 86.6%]	111 (55.0%) [48.1 - 61.7%]	---	50 (68.5%) [57.1 - 78.0%]	61 (47.3%) [38.9 - 55.9%]	---
≥ 1 CTC	35 (18.2%) [13.4 - 24.3%]	91 (45.0%) [38.3 - 51.9%]	0.0000	23 (31.5%) [22.0 - 42.9%]	68 (52.7%) [44.1 - 61.1%]	0.005
≥ 2 CTC	26 (13.5%) [9.4 - 19.1%]	79 (39.1%) [32.6 - 46.0%]	0.0000	20 (27.4%) [18.5 - 38.6%]	59 (45.5%) [37.4 - 54.3%]	0.011
≥ 3 CTC	15 (7.8%) [4.8 - 12.5%]	68 (33.7%) [27.5 - 40.4%]	0.0000	16 (21.9%) [14.0 - 32.7%]	52 (40.3%) [32.2 - 48.9%]	0.009

# CTCs Observed	Proportions of Subjects with Non-Normal Cells Observed in Harvests Deposited onto Wright-Giemsa Stained Cytology Slides					
	Evaluable HV Subjects	Evaluable MBC Subjects	Fisher's Exact p-value	Newly Diagnosed MBC Subjects	Recurring/Progressing MBC Subjects	Fisher's Exact p-value
≥ 4 CTC	10 (5.2%) [2.9 - 9.3%]	63 (31.2%) [25.2 - 37.9%]	0.0000	14 (19.2%) [11.8 - 29.7%]	49 (38.0%) [30.1 - 46.6%]	0.007
≥ 5 CTC	6 (3.1%) [1.4 - 6.6%]	58 (28.7%) [22.9 - 35.3%]	0.0000	12 (16.4%) [9.7 - 26.6%]	46 (35.7%) [27.9 - 44.2%]	0.004
≥ 10 CTC	1 (0.5%) [0.1 - 2.9%]	41 (20.3%) [15.3 - 26.4%]	0.0007	8 (11.0%) [5.6 - 20.2%]	33 (25.6%) [18.8 - 33.7%]	0.017
TOTAL N	192	202		73	129	

These results also show that MBC patients with recurring/progressive metastatic disease were found to have one or more CTCs or other abnormal cells at a higher proportion (observed on their Wright-Giemsa stained cytology slides) compared to MBC patients with newly diagnosed disease.

Molecular evaluation of Parsortix® PC1 cell Harvests

Material harvested from the ANG-002 study (HVs and MBC patients) was subjected to several standard representative molecular techniques currently used in clinical and/or research laboratory settings. Overall, the results obtained demonstrate that the Parsortix® PC1 system is capable of the capture and harvest of cells from the blood of metastatic breast cancer patients and the harvested cells can be analyzed with a number of methods used to evaluate the molecular signature of the circulating tumor cells present in the sample. Results from these studies further supported the claim that the Parsortix® PC1 device was shown to capture and harvest CTCs from a higher proportion of MBC patients compared to healthy volunteers. However, as noted earlier, with the current IU for the Parsortix® PC1, there are no claims associated with these representative downstream tests; any techniques/tests used in association with the Parsortix® PC1 device must be appropriately considered and validated by end users for purposes of evaluating isolated CTCs. The end user should determine whether the sample isolated is appropriate for downstream use, test or technology or if additional enrichment may be necessary.

N. Software:

1. Level of Concern: The Parsortix PC1 device was identified to have a moderate-level of concern as described in the FDA guidance document “Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices” (May 11, 2005). This determination was made since the software is an integral part of the Parsortix PC1 device.

2. Software Description:

The Parsortix® PC1 system is a standalone bench top laboratory instrument consisting of five main sub-system components:

- Parsortix PC1 instrument containing a computer, keypad and display, pneumatic and hydraulic components including reservoir bottles and tubes, a separation cassette mounting clamp and other electronics to control the instrument hardware and behavior.
- Parsortix PC1 Software consisting of a Windows 7 Embedded operating system together with dedicated Parsortix PC1 proprietary Windows application software (Software).
- A set of embedded and encrypted Protocol Files (Protocols) that are sequences of simple instructions, interpreted by the Software and used to control the instrument fluidic and hydraulic components and circuits. The Protocols supplied embedded within the Software enable the four core instrument processes: Clean, Prime, Separate, and Harvest.
- Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit which contains Separation Cassettes (n = 10, 50 or 100), Cleaning Cassettes [(n = 1, 5, or 10), one Cleaning Cassette for every multiple of 10 x separation cassette], Encrypted Instrument protocol file distributed on a USB memory stick as required to perform the proposed intended use.
- Parsortix PC1 ICT-01 Instrument Control Test Kit which contains Control tubes (n = 10 or 25) containing a known, aliquoted cell suspension which is used to periodically confirm acceptable performance of the system.

The Parsortix PC1 system software application (Application) has been developed to run as a standard Windows application on a PC compatible motherboard embedded in the PC1 instrument hardware configuration. The Application makes use of the Windows 7 Embedded operating system environment for low level functions including: memory management, file management, timers and timing functions and system hardware control. It controls the instrument user interface consisting of the keypad, LCD screen and buzzer.

The Windows 7 Embedded installation is specifically configured to automatically boot-up and execute the Parsortix Application with no user intervention. In this way, the system powers-up and is ready for intended operation without any requirement for user interaction. The software also includes a re-start mechanism to enable user recovery from fault conditions and unexpected instrument states without compromising overall integrity.

All other Windows and PC1 software configuration settings are pre-defined at the point of manufacture (software compilation) and not accessible to users or modifiable by users.

ANGLE technical support has limited access to configuration items to enable software upgrades and support instrument troubleshooting or maintenance and, using the Windows infrastructure, an external PC compatible keyboard and monitor can also be used to control the instrument.

The instrument is not intended to be connected to a local area network (LAN) and the LAN functionality and internet connectivity have been disabled at the point of manufacture.

3. Documentation associated with each of the software activities (e.g., Software Requirements Specification, Software Design Requirements, Device Hazard Analysis, etc.) described in the 2005 “Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices” for a Moderate Level of Concern, were provided and found to be adequate.

O. Proposed Labeling

The labeling supports the decision to grant the De Novo request for this device.

P. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Mitigation Measures
Failure to identify CTCs that are present in the sample leading to delays in patient management.	• Use of certain specimen collection devices identified in special control (1). • Certain labeling information identified in special control (2), including limitations, device descriptions, training specifications, explanation of procedures, and performance information identified in special control (3). • Certain design verification and validation identified in special control (3), including documentation of certain analytical studies and clinical studies.
No results obtained using downstream testing leading to delays in patient management.	• Certain labeling information identified in special control (2), including limitations, device descriptions, training specifications, explanation of procedures, and performance information identified in special control (3).
Incorrect evaluation of CTCs using downstream analyses leading to associated risk of false test results and improper patient management.	• Certain labeling information identified in special control (2), including limitations, device descriptions, explanation of procedures, and performance information identified in special control (3).

Identified Risks to Health	Mitigation Measures
Failure to correctly operate the device leading to delays in patient management and associated risk to downstream analyses resulting in false test results and improper patient management.	Certain labeling information identified in special control (2), including limitations, device descriptions, and explanation of procedures.
Bloodborne pathogen transmission from blood waste/blood sample.	Certain labeling information identified in special control (2), including limitations, device descriptions, and explanation of procedures.

Q. Benefit-Risk Assessment

Summary of the Assessment of Benefits:

The Parsortix® PC1 system is an in vitro diagnostic device intended to harvest CTCs from peripheral blood of patients diagnosed with MBC. Harvested CTCs can be used for subsequent downstream applications such as molecular, histopathological, and cytological techniques in common laboratory use or other FDA approved/cleared tests. The benefits of the device can be realized in situations where the aforementioned techniques or tests are used on such cells to enhance the understanding of the disease and accordingly supplement information already available in MBC patients without contributing to specific clinical decision-making in this context. Although the device as indicated is not by itself capable of identifying, enumerating, or characterizing CTCs, the benefit of the device can be realized in situations where tissue biopsy is not feasible and tumor material is otherwise not available and where the enrichment of such material may enhance the performance of the downstream testing that might be considered. The analysis of harvested CTCs can provide transcriptomic and proteomic information and allow morphological and cytological analysis, which cannot be provided by the evaluation of ctDNA. In conjunction with appropriate downstream assays, the ability to characterize and interrogate intact viable cells originating from tumors that have been harvested from the peripheral circulation (i.e., the cells provided by the Parsortix PC1 system) could provide additional background insights into tumor biology and the likelihood of some tumor cells to resist the therapies that might be considered to be administered in a similar patient population.

Summary of the Assessment of Risks

CTCs harvested with this device can be used for subsequent downstream applications. There is no immediate clinical use of the device on its own. Accordingly, in this context, it cannot be used to make clinical assessments or decisions. Its use is solely to enhance the understanding of the disease and accordingly supplement information that may already be available in MBC patients without contributing to clinical decision-making in this context. The end user should determine whether the sample isolated is appropriate for downstream use, test or technology or if additional enrichment may be necessary. Nonetheless, with

proper selection and use of downstream tests, there are risks related to the misidentification of nontumor cells as tumor cells, as well as the inability to use properly enriched tumor cells for such tests. In addition, selection of such FDA-cleared or approved tests may be incorrect for the particular clinical situation, and further, there is a possibility that such downstream tests may not be used for the approved intended use or may not be used according to the approved instructions for use.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

Summary of the Assessment of Benefit-Risk

Although there is probable benefit in certain clinical circumstances defined by the selected downstream tests for which the CTCs will be used, the benefits of the device cannot be realized when used on its own. Rather, the device can be used in conjunction with subsequent downstream applications to enhance the understanding of the disease and accordingly supplement information already available in MBC patients. In this clinical setting of metastatic breast cancer, the risks related to the misidentification of non-tumor cells as tumor cells, as well as the inability to use properly enriched tumor cells for properly selected downstream tests, are adequately mitigated by the special controls such that they are considered to be outweighed by the aforementioned benefits after taking into consideration the general controls and special controls.

R. Conclusion

The De Novo request is granted, and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QSA

Device type: Circulating tumor cell (CTC) enrichment device

Class: II

Regulation: 21 CFR 866.6110