Parsortix® PC1
MBC-01 Metastatic Breast Cancer Kit
Instructions for Use

Intended Use: The Parsortix® PC1 system is an in vitro diagnostic device intended to enrich circulating tumor cells (CTCs) from peripheral blood collected in K3EDTA 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.

www.angleplc.com/ivd-ifu

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1. **Intended Use**

**For In Vitro Diagnostic 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. **References**

The following documents are referenced:

- **PC1-OM-F Parsortix PC1 Instrument Instructions for Use**
- **ICT-OM-F Parsortix PC1 ICT-01 Instrument Control Test Kit Instructions for Use**

3. **Summary and Explanation**

The Parsortix PC1 instrument used in combination with the Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit (MBC-01 kit) enables the enrichment and isolation of CTCs from a peripheral blood sample drawn from metastatic breast cancer patients into K₂EDTA tubes and provides them as free cells suspended in buffer to allow a variety of subsequent downstream evaluations in accordance with Section 1 above. The Parsortix PC1 ICT-01 Instrument Control Test Kit (ICT-01 kit) is used periodically (weekly) in conjunction with the Parsortix PC1 instrument and the MBC-01 kit to verify acceptable Parsortix PC1 instrument performance. All three of these components (the Parsortix PC1 instrument, MBC-01 kit, and ICT-01 kit) comprise the Parsortix PC1 system (device). Refer to the Instructions for Use (IFU) for the MBC-01 and ICT-01 kits for additional information. These IFUs can be found here: [www.angleplc.com/ivd-ifu](http://www.angleplc.com/ivd-ifu)

4. **Principle of Operation**

The Parsortix PC1 instrument is intended for use by suitably trained users in a clinical laboratory setting and must be used in conjunction with the MBC-01 and ICT-01 kits. The MBC-01 kit enables the user to process a peripheral blood sample drawn into a K₂EDTA tube from a metastatic breast cancer patient on a Parsortix PC1 instrument for the capture and harvest of CTCs. The MBC-01 kit contains non-sterile, disposable, and single-use Parsortix PC1 GEN3P6.5IVD cell separation cassettes (separation cassettes), through which the blood is pushed across a continuous precision-molded separation structure with a final "critical gap" where CTCs are captured. Most of the typical blood cells (i.e., red blood cells, white cells, and platelets) pass through the "critical gap" while larger and less compressible cells, such as CTCs, are retained. The Parsortix PC1 instrument enables captured CTCs to be harvested from the cassette into an external vessel (e.g. a microfuge tube) for further, user-defined and validated subsequent downstream evaluation.

The MBC-01 kit also contains Parsortix PC1 GEN3C cleaning cassettes (cleaning cassettes) to facilitate the cleaning of the Parsortix PC1 instrument in-between blood processing cycles or after a period of >24 hours of non-use.
5. **Materials Provided**

The MBC-01 kit is provided for use with the Parsortix PC1 instrument to perform the device intended use as set out in Section 1 of this document.

The Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit contains the following items:

- $N$ Parsortix PC1 GEN3P6.5IVD Cell Separation Cassettes ($N$ is defined as the number of tests contained in a single kit, where $N = 10, 50, 100$);
- $M$ Parsortix PC1 GEN3C Cleaning Cassettes ($M$ is defined as one GEN3C cleaning cassette for every multiple of $10 \times N$ supplied in the kit, where $M = 1, 5, 10$);
- $X$ Sample label sets (Where $X = 10, 50, 100$ – e.g. Sets of labels, each set containing three individual labels to enable labeling of the sample tube, separation cassette and harvest vessel);
- One package insert (per kit) containing instructions for use and expected performance data for the Parsortix PC1 instrument, when used in conjunction with the Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit.

Each MBC-01 kit is sufficient to enable the processing 10, 50 or 100 blood samples when used in conjunction with the Parsortix PC1 instrument and the ICT-01 kit, as described in this document.

6. **Materials Required, Not Provided**

**Equipment:**

- Parsortix PC1 instrument pre-loaded with the application software and the following core protocol files: **MBC01**, **PX2_CLEAN**, **PX2_LOAD** and **PX2_PRIMING**.
- Calibrated 300µL pipettor
- Pipette-aid

**Reagents and consumables:**

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Manufacturer</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICT-01</td>
<td>ANGLE</td>
<td>Parsortix PC1 ICT-01 Instrument Control Test Kit</td>
</tr>
<tr>
<td>366643</td>
<td>BD</td>
<td>Plastic whole blood tube with spray-coated K$_2$EDTA</td>
</tr>
<tr>
<td>352098</td>
<td>BD/Falcon</td>
<td>50mL Falcon Tubes</td>
</tr>
<tr>
<td>143205WR</td>
<td>Steris Life Sciences</td>
<td>ProKlenz® 120</td>
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<td>Varies</td>
<td>100mL sterile serological pipettes</td>
</tr>
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<td>Varies</td>
<td>Sterile, filtered PBS (500mL Bottles) without Ca$^{2+}$/Mg$^{2+}$</td>
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<tr>
<td>Varies</td>
<td>Varies</td>
<td>Deionized Water (1 Liter Bottles)</td>
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<td>Varies</td>
<td>Varies</td>
<td>Absolute Ethanol (99.5%)</td>
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<tr>
<td>Varies</td>
<td>Varies</td>
<td>Clorox® Hypochlorite Bleach</td>
</tr>
<tr>
<td>Varies</td>
<td>Varies</td>
<td>Alcohol pre-soaked wipes</td>
</tr>
<tr>
<td>Varies</td>
<td>Varies</td>
<td>1.5mL Eppendorf tube (as harvest tube) or alternative harvest vessel</td>
</tr>
</tbody>
</table>
7. **Warnings and Precautions**

- **CAUTION:** Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices to use, or order the use of, the device.

- **WARNING:** Instructions for use and labeling for the Parsortix PC1 instrument, MBC-01 kit and ICT-01 kit must be followed at all times.

- **WARNING:** Following the Parsortix PC1 instrument, ICT-01 and MBC-01 kit Instructions for Use will ensure optimum device performance. It is the responsibility of the user to ensure instrument performance is adequate for specific downstream evaluation.

- **WARNING:** Do not use GEN3C cleaning cassettes in place of GEN3P6.5IVD cell separation cassettes for the processing of blood samples on the Parsortix PC1 instrument.

- **WARNING:** Blood sample processing should not be conducted as per the MBC-01 kit Instructions for Use using a Parsortix PC1 instrument that has not undergone the weekly maintenance, including a successful ICT procedure as described in the ICT-01 kit Instructions for Use.

- **WARNING:** The Parsortix PC1 instrument together with the ICT-01 and MBC-01 kits is designed for use in a high complexity testing laboratory environment, as defined by CLIA. It must be situated and operated only in facilities with the specialized infrastructure and general equipment required for clinical laboratory operations, including those with blood disposal facilities following universal precautions. Users must follow these universal precautions and use specified laboratory safety equipment. All chemicals and all consumables that had contact with blood must be disposed of using adequate precautions and in accordance with local, state, and national regulations.

- **WARNING:** Dependent on patient condition, a blood sample processed on a Parsortix PC1 instrument together with the MBC-01 kit may not include a sufficient number of circulating tumor cells for a particular downstream application, which may impact the success of any subsequent evaluation.

- **WARNING:** The Parsortix PC1 GEN3C cleaning cassettes and GEN3P6.5IVD cell separation cassettes (collectively cassettes) are fragile and must be handled with care. Always handle cassettes by the edges and avoid applying pressure to its surfaces.

- **WARNING:** Follow Health and Safety and Precautionary statements as described in the Parsortix PC1 Instrument Instructions for Use.
8. Limitations

- For In Vitro Diagnostic Use

- The standalone device, as indicated, is not intended for cell enumeration.

- The Parsortix PC1 Instrument must only be used in conjunction with a Parsortix PC1 ICT-01 Instrument Control Test Kit (ICT-01 kit) and a Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit (MBC-01 kit) and in accordance with the Intended Use set out in the instructions accompanying the equipment and kits.

- The variability of the number of CTCs and other cells harvested by the device, including insufficient number of CTCs or even no CTCs collected, may impact the success of any subsequent analysis.

- The performance characteristics of this device have not been established for general downstream diagnostic assays and end users must validate use with any subsequent tests and collection devices.

- The standalone device, as indicated, is not intended for diagnostic, prognostic, or monitoring use with circulating tumor cells, including as an aid in any disease management and/or treatment decisions.

- The use of the device is indicated for previously diagnosed metastatic breast cancer patients.

- Results from the standalone device, as indicated, do not provide information to the patient regarding their current state of health.

- The standalone device, as indicated, does not diagnose any health conditions and is not a substitute for visits to a doctor or other healthcare professional.

- The standalone device provides material (circulating tumor cells) that must be processed/analyzed using additional independent methods (referred to as subsequent downstream assays). Subsequent downstream assays must be validated by the end user, as needed.

- CTC identification or enumeration is not provided by the standalone device as indicated. The Parsortix PC1 device, as indicated, is intended for use on patients already diagnosed with metastatic breast cancer. No clinical decisions (diagnostic, prognostic, predictive or any treatment) should be made solely on the basis of the output obtained with the Parsortix PC1 device. It is the responsibility of the user to ensure validation of the downstream analysis is completed in conjunction with other FDA approved assays/tests and/or in accordance with CLIA laboratory requirements before clinical assessment.

- Significant numbers of white blood cells (WBCs) may also be present in the output material provided by the Parsortix PC1 system (device). It is the responsibility of the user to ensure that this does not interfere with specific downstream assays.

- High hematocrit levels increase the sample processing time and moderately increase the number of residual WBCs in the harvest while lower hematocrit levels significantly increase the average number of nucleated blood cells in the harvest.

- The presence of ~80µg/mL of Paclitaxel in blood was found to potentially have an impact on the functioning of the Parsortix system which may cause the loss of samples and/or reduction in the quality of the output material (harvest).
9. **Storage and Handling**

9.1 **Storage**

The MBC-01 kit content is supplied ready for use. The pack must be visually inspected for any sign of damage before use. The Parsortix PC1 GEN3P6.5IVD cell separation cassettes must be stored at room temperature protected from exposure to sunlight. When properly stored, cassettes are stable until the expiration date printed on the packaging. Do not use expired cassettes.

9.2 **Handling**

For instructions on how and when to load and remove cassettes on and from the Parsortix PC1 instrument, and for reagent handling required to operate the instrument, please refer to the *PC1-OM-F Parsortix PC1 Instrument Instructions for Use*.

The MBC01 protocol will be installed on the Parsortix PC1 instrument during instrument set-up by trained ANGLE personnel.

10. **Sample Processing Procedure**

10.1 **Workflow Overview**

*Figure 1* below shows the current typical operating workflow sequence for the Parsortix PC1 instrument when the MBC01 protocol is executed. The individual steps of the MBC01 protocol and their respective functions are as follows:

- **Prime** – the application of fluid to eliminate air bubbles from the fluidic system and prime the system and a new GEN3P6.5IVD separation cassette ready to receive samples and/or reagents (Time required: ~10 minutes);

- **Blood Separation** – the movement of a sample of blood (drawn into the specified K2EDTA Vacutainer® and attached to the instrument) from the blood sample tube through the GEN3P6.5IVD separation cassette to perform cell separation and capture within the cassette and 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 (Time required: ~2 hours, but is blood volume/patient dependent);

- **Cell Harvest** – Internal instrument tubing is flushed to remove residual blood cells and then the cells captured in the separation cassette are eluted into an external vessel for further analyses (Time required: ~15 minutes);

- **Clean** – the application of cleaning fluid to remove contamination, debris and residual reagents from the system and buffer to rinse the cleaning fluid from the system (Time required: ~40 minutes).
10.2 Specimen Collection and Preparation

Blood for processing on the Parsortix PC1 instrument with the MBC-01 kit must be drawn into a K\textsubscript{2}EDTA Becton Dickinson 10mL Vacutainer\textsuperscript{®}, product number 366643 (US). Acceptable blood volumes for processing are between 5mL and 10mL. Blood samples should be stored at room temperature and processed within 8 hours of collection. The patient (blood donor) must not have had any cytotoxic therapy administered during the previous 7 days.

10.3 Initial Checklist

Refer to *PC1-OM-F Parsortix PC1 Instrument Instructions for Use* document for full instructions on how to operate the Parsortix PC1 instrument. The following conditions need to be fulfilled before processing a blood sample:

- Visual inspection of the external instrument tubing for any signs of kinks, leaks, or damage.
- The reagents required for normal operation are in place according to *PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.5*.
- The Parsortix PC1 instrument must have passed the weekly maintenance procedure *PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.9*.
- The Parsortix PC1 instrument must have been cleaned after the last operation and within the past 24 hours prior to use. Refer to *PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.8.2* for cleaning instructions.
- The amount of liquid in the waste reagent bottle must be less than 400mL. Otherwise, follow the instructions as per *PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.5.1*.
- A clean 50mL Falcon tube is attached to the sample mount of the Parsortix PC1 instrument.
A GEN3C cleaning cassette is correctly inserted in the cassette clamp. Refer to PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.4 for cassette handling instructions.

The harvest valve is turned clockwise to the “SEP” position (PC1-OM-F Parsortix PC1 Instrument Instructions for Use Section 10.6).

The harvest waste line “H” is plugged into the cap of the harvest waste tube.

A new Parsortix PC1 GEN3P6.5IVD cell separation cassette is available.

An additional empty 50mL Falcon tube is available.

10.4 Sample Processing with the Parsortix PC1 Instrument

1. In the main menu screen, select the protocol "MBC01“ and press [Run] then [Start] and follow the on-screen instructions.

2. At prompt “100mL Buffer?” check that the buffer reservoir (250mL bottle connected to the line labeled “B”) contains >100mL of PBS. Press [OK].

3. At prompt “50mL ProKlenz?” check that the cleaning reagent reservoir (250mL bottle connected to the line labeled “C”) contains >50mL of 10% ProKlenz solution. Press [OK].

4. At prompt “10mL EtOH?” check that the priming reagent reservoir (100mL bottle connected to the line labeled "P") contains >10mL 100% (absolute) ethanol. Press [OK].
5. At prompt “Waste empty?” ensure that the waste reservoir (500mL bottle connected to the line labeled “W”) has <400mL of liquid. If the level of the waste fluid is ≥ 400mL, change the waste reservoir as detailed in *PC1-OM-F Parsortix PC1 Instrument Instructions for Use* Section 10.5.1. Press [OK].

6. At prompt “Insert New Cassette” “Ready?”, open the cassette clamp and remove the GEN3C cleaning cassette, keeping it safe. Insert a new, properly labeled, GEN3P6.5IVD separation cassette into the cassette clamp. Close the clamp and press [OK].

7. At prompt “Rinse Vacutainer” “Start?”, pull the currently mounted 50mL Falcon tube off of the sample mount, keeping the sample line inside the tube. Press [OK] to start the rinse procedure and collect the fluid dispensed by the instrument inside the tube. At the end of the rinse procedure, fully remove the 50mL Falcon tube and use an alcohol-soaked wipe to carefully wipe the outside of the sample line and the O-rings on the sample mount.
8. At prompt “Preparing sample” “Attach Vacutainer…”, invert the properly labeled blood sample vacutainer 5 times, remove the stopper, and attach it onto the sample mount of the instrument. A twisting action will help to push the mouth of the tube over the O-ring. Secure the sample tube by pushing it into the vertical position and press [OK].
9. At prompt "Preparing Sample" "Start?" press [OK] to start the separation of the blood sample.

10. After 30 minutes, gently pull the sample tube into the incline position and tap the bottom of the sample tube while it sits in the inclined position to re-suspend the settled blood cells. Secure the sample tube by pushing it back into the vertical position. Repeat this step every 30 minutes.

11. At prompt "Rotate Valve..." "Counterclockwise", rotate the harvest valve counterclockwise into the HAR position. Press [OK]. Check that the sample tube is empty and that the GEN3P6.5IVD separation cassette is clear of blood.
12. At prompt “Cell recovery” “Start?”, remove the harvest line from the harvest waste tube and clean it with an alcohol-soaked wipe. Place a properly labeled collection vessel (e.g. 1.5mL Eppendorf tube) beneath the harvest line, and press [OK] to harvest the cells captured in the GEN3P6.5IVD separation cassette into the collection vessel. Make sure drops of liquid are being dispensed into the harvest vessel.

13. At prompt “Rotate Valve…” “Clockwise”, place the harvest line back into the harvest waste tube and rotate the harvest valve clockwise to the SEP position. Press [OK].

14. At prompt “End of Harvest” “Insert Cleaning Cass”, open the cassette clamp, remove the GEN3P6.5IVD separation cassette and dispose of it safely. Insert the GEN3C cleaning cassette into the clamp, close the clamp assembly and press [OK]. The cleaning cycle will take approximately 45 minutes to complete.

15. At prompt “Remove Vacutainer”, carefully remove and properly dispose of the mounted sample tube and press [OK]. A twisting motion will help with the removal of the sample tube.
16. At prompt “CleanVacutainer-line”, clean the O-rings on the sample mount and the outside of the sample line using an alcohol-soaked wipe. Press [OK].

17. At prompt “Attach newVacutainer”, place a clean 50mL Falcon tube onto the sample mount. Empty the fluids from the harvest waste tube and reattach the tube to the harvest line. Press [OK].

18. At prompt “Finished MBC01”, press [OK] then [Continue] to return to the main menu screen.
11. Output

The output of the process is a suspension of cells harvested from a GEN3P6.5IVD separation cassette in 210μL of PBS buffer for further downstream evaluation. No other output is provided using the Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit and the Parsortix PC1 instrument.

12. Quality Control

Acceptable Parsortix PC1 instrument performance must be established on a weekly basis prior to the processing of blood samples. The Parsortix PC1 ICT-01 Instrument Control Test Kit must be used as part of the specified weekly maintenance procedure (refer to PC1-OM-F Parsortix PC1 Instrument Instructions for Use and to ICT-OM-F Parsortix PC1 ICT-01 Instrument Control Test Kit Instructions for Use). Please refer to ICT-OM-F Parsortix PC1 ICT-01 Instrument Control Test Kit Instructions for Use for expected values of the test procedure.

13. Interpretation of Results

CTC identification or enumeration is not provided by the Parsortix PC1 instrument nor the Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit. The Parsortix PC1 device is intended for use on patients already diagnosed with metastatic breast cancer. No clinical decisions (diagnostic, prognostic, predictive or any treatment) should be made solely on the basis of the output obtained using the Parsortix PC1 instrument and Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit. Subsequent downstream evaluation of the output obtained from the device is required to enable any clinical decisions. It is the responsibility of the user to ensure validation of the downstream analysis is completed in conjunction with other FDA approved assays/tests and/or in accordance with CLIA laboratory requirements before clinical assessment.
14. Expected Performance

14.1 Cell Recovery Studies

14.1.1 Live SKBR3 Cells Spiked into Blood

Eight 7.5mL blood samples from a single healthy female donor were spiked with 2, 5, 10, 15, ~25 (24-26), ~50 (48-52), ~75 (72-78) and ~100 (95-105) live and fluorescently pre-labeled cultured breast cancer cells (SKBR3). In addition, four 7.5mL blood samples from a single healthy female donor were spiked with approximately 125, 250, 500, and 1000 live and fluorescently pre-labeled cultured breast cancer cells (SKBR3). These samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the number of recovered spiked pre-labeled cells counted using a fluorescence microscope. The experiment was repeated for nine additional female donors. The counts of recovered cells of the combined sets of samples were plotted against the expected cell counts and results are summarized in Table 1.

Table 1. Percent Recovery Estimates – Live SKBR3 Cells spiked into blood

<table>
<thead>
<tr>
<th>Mean Actual Cell Count</th>
<th>Mean Recovered Cell Count</th>
<th>Mean Percent Recovery</th>
<th>Range of Percent Recovery</th>
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<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>70%</td>
<td>0% to 100%</td>
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<td>5</td>
<td>4</td>
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<td>43% to 78%</td>
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<tr>
<td>489</td>
<td>331</td>
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</tr>
<tr>
<td>1113</td>
<td>729</td>
<td>66%</td>
<td>55% to 77%</td>
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The best fit model for the combined live SKBR3 cell linearity evaluation described above was determined to be the first order linear regression model (Figure 2), indicating that the Parsortix PC1 system was linear over the range of 2 to ~1,000 live SKBR3 cells spiked into 7.5mL of blood. The linear model had a slope of 0.6543, indicating an average recovery rate of ~65% (95% CI = 64% - 67%, R^2 = 0.9805) over the range of 2 to ~1,000 live SKBR3 cells.
Figure 2. Plot of Linearity Evaluation Results using Combined Live SKBR3 Cell Linearity Data. The figure shows a plot of the actual numbers of live SKBR3 cells spiked (X-axis) vs. the numbers of live SKBR3 cells harvested for each of the 10 replicates at each of the spiking levels. The equations for the first (indicated in black), second (indicated in blue) and third (indicated in burnt orange) order polynomial models, as well as the trendlines for each model, are also indicated on the graph.

14.1.2 Live MCF7 Cells Spiked into Blood

Eight 7.5mL blood samples from a single healthy female donor were spiked with 2, 5, 10, 15, ~25 (24-26), ~50 (48-52), ~75 (72-78) and ~100 (95-105) live and fluorescently pre-labeled cultured breast cancer cells (MCF7). These samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the number of recovered spiked pre-labeled cells counted using a fluorescence microscope. The experiment was repeated for nine additional female donors. The counts of recovered cells were plotted against the expected cell counts and results are summarized in Table 2.

Table 2. Percent Recovery Estimates – Live MCF7 Cells spiked into blood

<table>
<thead>
<tr>
<th>Mean # Cells Spiked</th>
<th>Mean # Cells Recovered</th>
<th>Mean Percent Recovery</th>
<th>Range of Percent Recovery</th>
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<td>46% to 83%</td>
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<td>69%</td>
<td>51% to 85%</td>
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<td>58</td>
<td>77%</td>
<td>56% to 93%</td>
</tr>
<tr>
<td>100</td>
<td>76</td>
<td>75%</td>
<td>66% to 83%</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>73%</td>
<td>0% to 150%</td>
</tr>
</tbody>
</table>
The best fit model for the live MCF7 cell linearity evaluation was determined to be the first order linear regression model (Figure 3), indicating that the Parsortix PC1 system was linear over the range of 2 to ~100 live MCF7 cells spiked into 7.5mL of blood. The linear model had a slope of 0.7581 indicating an average recovery rate of ~76% (95% CI = 73% - 79%, R^2 = 0.9716) over the range of 2 to ~100 live MCF7 cells.

**Figure 3. Plot of Linearity Evaluation Results using Live MCF7 Cell Linearity Data.** The figure shows a plot of the actual numbers of live MCF7 cells spiked (X-axis) vs. the numbers of live MCF7 cells harvested for each of the 10 replicates at each of the spiking levels. The equations for the first (indicated in black), second (indicated in blue) and third (indicated in burnt orange) order polynomial models, as well as the trendlines for each model, are also indicated on the graph.

### 14.1.3 Live Hs 578T Cells Spiked into Blood

Eight 7.5mL blood samples from a single healthy female donor were spiked with 2, 5, 10, 15, ~25 (24-26), ~50 (48-52), ~75 (72-78) and ~100 (95-105) live and fluorescently pre-labeled cultured breast cancer cells (Hs 578T). These samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the number of recovered spiked pre-labeled cells counted using a fluorescence microscope. The experiment was repeated for nine additional female donors. The counts of recovered cells were plotted against the expected cell counts and results are summarized in Table 3.

**Table 3. Percent Recovery Estimates – Live Hs 578T Cells spiked into blood**

<table>
<thead>
<tr>
<th>Mean Actual Cell Count</th>
<th>Mean Recovered Cell Count</th>
<th>Mean Percent Recovery</th>
<th>Range of Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>90%</td>
<td>0% to 100%</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>80%</td>
<td>40% to 100%</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>76%</td>
<td>60% to 120%</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>76%</td>
<td>53% to 93%</td>
</tr>
<tr>
<td>26</td>
<td>20</td>
<td>77%</td>
<td>64% to 96%</td>
</tr>
<tr>
<td>51</td>
<td>41</td>
<td>80%</td>
<td>62% to 90%</td>
</tr>
<tr>
<td>77</td>
<td>60</td>
<td>78%</td>
<td>59% to 94%</td>
</tr>
<tr>
<td>100</td>
<td>76</td>
<td>76%</td>
<td>64% to 82%</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>79%</strong></td>
<td><strong>0% to 120%</strong></td>
<td></td>
</tr>
</tbody>
</table>
The best fit model for the live Hs 578T cells was determined to be the first order linear regression model (Figure 4), indicating that the Parsortix PC1 system was linear over the range of 2 to ~100 live Hs 578T cells spiked into 7.5mL of blood. The linear model had a slope of 0.7654 indicating an average recovery rate of ~76% (95% CI = 74% - 79%, R² = 0.9791) over the range of 2 to ~100 live Hs 578T cells.

**Figure 4. Plot of Linearity Evaluation Results using Live Hs 578T Cells Linearity Data.** The figure shows a plot of the actual numbers of live Hs 578T cells spiked (X-axis) vs. the numbers of live Hs 578T cells harvested for each of the 10 replicates at each of the spiking levels. The equations for the first (indicated in black), second (indicated in blue) and third (indicated in burnt orange) order polynomial models, as well as the trendlines for each model, are also indicated on the graph.

### 14.1.4 Linearity of Live vs. Fixed SKBR3 Cells

Eight 7.5mL blood samples from a single healthy female donor were spiked with 2, 5, 10, 15, ~25 (24-26), ~50 (48-52), ~75 (72-78) and ~100 (95-105) fixed and fluorescently pre-labeled cultured breast cancer cells (SKBR3). These samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the number of recovered spiked pre-labeled cells counted using a fluorescence microscope. The experiment was repeated for nine additional female donors. The counts of recovered cells were plotted against the expected cell counts and results are summarized in Table 4.

**Table 4. Percent Recovery Estimates – Fixed SKBR3 Cells**

<table>
<thead>
<tr>
<th>Mean Actual Cell Count</th>
<th>Mean Recovered Cell Count</th>
<th>Mean Percent Recovery</th>
<th>Range of Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>85%</td>
<td>50% to 100%</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>86%</td>
<td>60% to 100%</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>94%</td>
<td>80% to 110%</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>91%</td>
<td>80% to 100%</td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>93%</td>
<td>80% to 100%</td>
</tr>
<tr>
<td>51</td>
<td>46</td>
<td>91%</td>
<td>82% to 102%</td>
</tr>
<tr>
<td>76</td>
<td>68</td>
<td>89%</td>
<td>80% to 96%</td>
</tr>
<tr>
<td>101</td>
<td>90</td>
<td>89%</td>
<td>56% to 100%</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td><strong>90%</strong></td>
<td><strong>50% to 110%</strong></td>
</tr>
</tbody>
</table>
The harvesting of both live and fixed SKBR3 cells spiked into blood by the Parsortix PC1 system was demonstrated to be linear from 2 to ~100 cells (whole interval evaluated). For both the live and fixed pre-labeled SKBR3 cell datasets, the best fit models were determined to be the first order linear regression models (shown in Figure 5 below), allowing for a direct comparison of the derived linear models. As illustrated in Figure 5, the Parsortix PC1 system was able to recover a significantly higher proportion of fixed SKBR3 cells spiked into 7.5mL of 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.

Figure 5. Comparison of linearity on Parsortix PC1 System using live vs. fixed pre-labeled SKBR3 cells spiked into 7.5mL of blood over a range of 2 to ~100 cells and processed using Parsortix PC1 Systems where captured cells were harvested directly into 96-well plates for identification.

The results from all of the linearity studies above show that live pre-labeled SKBR3, MCF7 and Hs 578T cells, as well as fixed SKBR3 cells, are harvested by the Parsortix PC1 system in a linear fashion when the number of cells spiked into 7.5mL of blood ranges from 2 to ~100 cells (Figure 6).
**14.2 Detection Limit**

The detection limit is defined as the minimum number of live, fluorescently labeled and spiked tumor cells in a 7.5mL blood sample required to recover at least one spiked tumor cell using the Parsortix PC1 system ≥95% of the time. The detection limit of the Parsortix PC1 system was determined by spiking a range of breast cancer cell lines (SKBR3, Hs 578T and MCF7) into a minimum of sixty (60) 7.5mL healthy donor blood samples for each cell line separately.

The established detection limit of the Parsortix PC1 system ranged between 3 and 5 cells depending on the cancer cell line used in this study (Table 5).

**Table 5. Detection Limit of the Parsortix PC1 system**

<table>
<thead>
<tr>
<th>Breast Cancer Cell Line Used</th>
<th>Detection Limit (to capture ≥1 cell, ≥95% of time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>3 Cells</td>
</tr>
<tr>
<td>Hs 578T</td>
<td>4 Cells</td>
</tr>
<tr>
<td>MCF7</td>
<td>5 Cells</td>
</tr>
</tbody>
</table>

The Limit of Blank of the Parsortix PC1 system is zero (0) cells.
14.3 Reproducibility and Repeatability

14.3.1 System Reproducibility with Live and Fixed Cancer Cells

7.5mL blood samples from two healthy donors were spiked with approximately 20 live and fluorescently labeled cultured breast cancer cells (SKBR3). The two samples were processed using Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the number of fluorescently labeled cells counted using a fluorescence microscope and plotted against the expected cell count. To generate the required data for the reproducibility analysis, the experiment was repeated on ten different Parsortix PC1 instruments over 20 days with two run sets per day, creating 400 datapoints. The overall average harvest percent was 70.4%, the repeatability %CV was 21.1% and the within-laboratory %CV was 22.0%.

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

14.3.2 System Lot-to-Lot Reproducibility

Twenty (20) fixed and fluorescently labeled cultured breast cancer cells (SKBR3) suspended in 1% BSA, 2mM EDTA PBS solution were used to assess the reproducibility of the Parsortix PC1 system using three different lots of Parsortix GEN3 cell separation cassettes (6.5µm critical gap). Samples were separated using ten (10) Parsortix PC1 instruments during two runs per instrument per day over ten days for each cassette lot tested, generating 200 measurements per cassette lot. 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%.

14.3.3 System Reproducibility Across Sites

Twenty (20) fixed and fluorescently labeled cultured breast cancer cells (SKBR3) suspended in 1% BSA, 2mM EDTA PBS solution were used to assess the reproducibility of the Parsortix PC1 system across three different sites (ANGLE R&D Center (Guildford), UK; MD Anderson Cancer Centre (MDA), USA; University of Rochester Medical Center (URMC), USA). Samples were separated using a total of 20 instruments across the three sites during two runs per instrument per day over a total of 20 days, generating a total of 800 datapoints. The overall average harvest percentages at the individual sites ranged from 65.4% to 81.6%, 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%.

Figure 7 below shows a data summary of all the different reproducibility studies carried out for the Parsortix PC1 system.
5-day single site precision: Additional single site 5-day precision studies were also performed. This was 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 6 below.
Table 6. Summary of Additional 5-Day Live-Cell Precision Studies

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Spiked cell counts description</th>
<th>N</th>
<th>Mean % Harvest (Repeatability) SD CV%</th>
<th>Between Run SD %CV</th>
<th>Between Day SD %CV</th>
<th>Within Laboratory SD %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live SKBR3</td>
<td>5</td>
<td>100</td>
<td>73.6% 22.8% 31.0%</td>
<td>6.7% 9.1% 0.0% 0.0%</td>
<td>4.4% 6.3% 19.1% 27.1%</td>
<td>23.7% 32.2%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>70.4% 18.5% 26.3%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>13.8% 19.1%</td>
</tr>
<tr>
<td></td>
<td>~50 (48-52)</td>
<td>100</td>
<td>72.1% 13.8% 19.1%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>13.8% 19.1%</td>
</tr>
<tr>
<td>Live MCF7</td>
<td>5</td>
<td>100</td>
<td>68.0% 22.0% 32.4%</td>
<td>7.3% 10.7% 1.3%</td>
<td>1.9% 23.2% 34.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>68.4% 18.4% 26.9%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>19.5% 28.5%</td>
</tr>
<tr>
<td></td>
<td>~50 (48-52)</td>
<td>100</td>
<td>76.2% 11.3% 14.8%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>11.8% 15.5%</td>
</tr>
<tr>
<td>Live Hs578T</td>
<td>5</td>
<td>100</td>
<td>66.6% 19.9% 29.9%</td>
<td>5.9% 8.9% 3.6%</td>
<td>5.4% 21.0% 31.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>63.5% 17.8% 28.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>19.3% 30.4%</td>
</tr>
<tr>
<td></td>
<td>~50 (48-52)</td>
<td>100</td>
<td>70.0% 8.6% 12.3%</td>
<td>0.0% 0.0% 0.0%</td>
<td>0.0% 0.0% 0.0%</td>
<td>9.3% 13.3%</td>
</tr>
</tbody>
</table>

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 Hs578T precision samples processed on Parsortix PC1 systems combined (spike levels between 5 - ~50 cells) were 25% and 26%, respectively.

14.4 Blood Volume

The impact of the volume of blood samples on the performance of the Parsortix PC1 system was evaluated by spiking a known number of fluorescently labeled, live cultured breast cancer cells (SKBR3) in 5mL, 7.5mL, and 10mL healthy female donor blood samples. Samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the numbers of pre-labeled cells counted using a fluorescence microscope. The number of cells counted in the harvest was plotted against the number of cells spiked for each sample. Results from these three blood sample sizes show that blood volume had no impact on the efficiency of the Parsortix PC1 system to capture and harvest target cells. Samples with higher blood volumes, as expected, required a longer processing time and resulted in a slightly, and statistically insignificant, higher number of residual nucleated cells being present in the Parsortix PC1 system harvests.

14.5 Blood Stability

Fluorescently labeled and cultured, live breast cancer cells (SKBR3) were spiked into 7.5mL blood samples collected in K2EDTA tubes, and the spiked samples were stored at room temperature and 4°C for up to 72 hours in order to evaluate the effect of storage conditions on the performance of the Parsortix PC1 system. The samples were processed on Parsortix PC1 systems, the captured cells harvested into 96-well plates, and the numbers of pre-labeled cells counted using a fluorescence microscope. The number of cells counted in the harvest was plotted against the number of cells spiked for each sample. Storing spiked blood samples at room temperature or 4°C for up to 72 hours had no significant impact on the capture or harvest of SKBR3 cells by the Parsortix PC1 system.
Furthermore, storing blood samples at room temperature for more than 24 hours after collection and before processing on the Parsortix PC1 system may result in significantly longer processing times while storage at 4°C for up to 72 hours had no significant impact on processing time.

Storing spiked blood samples at room temperature for more than 4 hours or at 4°C for more than 48 hours after collection and before processing will result in significantly higher numbers of residual nucleated cells in the harvests of the Parsortix PC1 system. The impact of residual nucleated cells in the Parsortix PC1 harvest on any downstream analysis requires specific evaluation by the end-user.

14.6 Cell Carryover

Two blood samples which were spiked with a high number (~1,000) of fluorescently labeled, live SKBR3, Hs578T, and MCF7 cells were processed on the Parsortix PC1 system followed by five 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 systems. Parsortix PC1 harvests from the PBS samples were examined for the presence of any pre-labeled cells or nucleated cells carried over within the instrument from the previous blood sample runs. Of the 220 PBS harvests, none showed a single carried-over fluorescently labeled cell, suggesting that the established cleaning procedure of the Parsortix PC1 system between sample runs ensures the absence of any cell carryover between samples.

14.7 Interfering Substances

14.7.1 Exogenous Substances

Fluorescently labeled, cultured live, breast cancer cells (SKBR3) spiked into blood samples were subjected to potential interfering cancer drugs 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, 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 Parsortix PC1 system ability to capture and harvest target cells.

None of the above tested drugs had an impact on the functioning of the Parsortix PC1 system except for Paclitaxel. The presence of ~80µg/mL of Paclitaxel in blood was found to potentially have an impact on the functioning of the Parsortix system which may cause the loss of samples and/or reduction in the quality of the harvest. Only one out of the twelve Paclitaxel samples (8%) was lost in this study due to sedimentation of the blood in the intake tubing of the Parsortix PC1 instrument during processing.

At the concentrations used, the drugs tested did not have an impact on the average number of nucleated blood cells (mostly white blood cells) present in the harvest except for Paclitaxel and Tamoxifen. Both drugs significantly increased the average number of nucleated cells present in the harvest. The impact of residual nucleated cells in the Parsortix PC1 harvest on any subsequent downstream analysis requires specific evaluation by the end user.

14.7.2 Endogenous Substances

Albumin

Higher concentration of albumin in the blood is not expected to interfere with the Parsortix PC1 system to capture and harvest target cells. When a high albumin level (~56 mg/mL) was induced in blood samples
spiked with live, fluorescently labeled SKBR3 cells, this condition did not have an impact on the number of harvested cells or the sample processing time when compared to control samples.

**Triglycerides**
Higher triglycerides level in the blood is not expected to interfere with the ability of the Parsortix PC1 system to capture and harvest target cells. When a high level of triglycerides (~500 mg/dL) was induced in blood samples spiked with live, fluorescently labeled SKBR3 cells, this condition did not have an impact on number of harvested cells or the sample processing time when compared to control samples.

**Hematocrit**
Live, fluorescently labeled SKBR3 cells were spiked in blood samples with different Hematocrit levels and processed on the Parsortix PC1 system. Different Hematocrit levels do not interfere with the ability of the Parsortix PC1 system to capture and harvest target cells. However, higher Hematocrit levels increased the sample processing time and moderately increased the number of residual white blood cells in the harvest whilst lower hematocrit levels significantly increased the average number of nucleated blood cells in the harvest.

**White blood cells**
Live, fluorescently labeled SKBR3 cells were spiked in blood samples where the number of white blood cells was modified to mimic blood samples with elevated white blood cell counts. High white blood cell counts (up to an average of 16x10^9 cells/L) were not found to interfere with the efficiency of the Parsortix PC1 system to capture and harvest SKBR3 cells.

However, elevated white blood cell levels led to a significant increase in the average number of nucleated blood cells harvested by the Parsortix PC1 system and may potentially lead to an increase in cells remaining in the separation cassette following the harvest of captured cells. The true impact of high white blood cell counts in a blood sample on sample processing time could not be established and the impact of white blood cell counts higher than the levels tested in this study (20.5x10^9 cells/mL) is unknown.

### 14.8 Evaluation of the Parsortix PC1 Device with Clinical Samples

Results from two separate studies were provided to support the performance of the Parsortix PC1 device with clinical samples.

In interpreting these results, it should be noted that the patient condition could impact the presence or absence of CTCs and this may change over time. CTCs have been shown to be involved in the progression of cancer, and thus patients whose disease is progressing are more likely to have CTCs present.

It should also be noted that the process of transferring cells to a microscope slide and then staining them for identification can result in cells being lost.

#### 14.8.1 Study #1 (ANG-008)
In this study, two K2EDTA tubes were collected from 76 healthy volunteer (HV) subjects and 77 metastatic breast cancer (MBC) patients.

**Primary Evaluation:** The tube from each subject with the largest volume of blood was spiked with ~20 fixed, CellTracker™ Green labelled SKBR3 cells. The spiked samples were processed on Parsortix PC1 systems using Parsortix GEN3 cell separation cassettes (6.5µm critical gap), and captured cells were harvested directly onto glass microscope slides. The number of pre-labelled SKBR3 cells harvested onto each slide was determined using fluorescence microscopy. The overall average percentage of spiked SKBR3
cells harvested from the 76 evaluable HV subject blood samples and the 74 evaluable MBC patient blood samples combined was 70.0% ± 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 subject blood samples was 72.1% ± 16.1% (Wilson 95% CI = 61.1% to 80.9%, median = 75.0%) compared to 67.9% ± 14.3% (Wilson 95% CI = 56.7% to 77.4%, median = 68.4%) in the 74 evaluable MBC patient blood samples (t-test p-value = 0.0981).

Secondary Evaluation: Using the remaining tube from each subject, the ability of the Parsortix PC1 system to capture and harvest CTCs (as identified by an immunofluorescence [IF] staining method) from the blood of HV subjects and MBC patients was assessed. The IF staining method used was limited to identification of CTCs with an epithelial phenotype and did not characterise additional mesenchymal CTCs or those in transition from epithelial to mesenchymal phenotype. The cells harvested from the blood samples processed for the IF evaluation were deposited onto charged glass cytology slides (Cytospin™ slides) using a cytocentrifugation method. Following fixation, the slides were stained using antibodies for CKs 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. The proportions of HV subjects and MBC patients with one (1) or more CTCs (cells that were DAPI positive, CD marker negative, and EpCAM and/or cytokeratin (CK) positive) identified on the IF-stained cytology slides using fluorescence microscopy were determined and compared.

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

The secondary evaluation 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 identified 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 identified as naked nuclei.

14.8.2 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 metastatic breast cancer (MBC).

The primary objectives of the study 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;
- Show that the harvested cells could be used in subsequent evaluations;
- Determine the proportion of MBC patients and healthy volunteers (HVs) that had one or more CTCs identified by a qualified pathologist using cytological evaluation of the harvested cells.

The secondary objective was to demonstrate the use of several different downstream evaluations designed to detect and/or characterize CTCs in the population of cells harvested by the device.
A total of 204 eligible HV subjects and 207 eligible MBC patients were enrolled into the study. A total of 192 HVs and 202 MBC patients had evaluable results from the cytological evaluation for the primary objectives while 198 HVs and 193 MBC patients had evaluable results from the secondary molecular evaluations.

**Cytological Evaluation:** An evaluation of the cytocentrifugation method used for the preparation of the cytology slides in this study was conducted to assess the efficiency (i.e. cell retention) and linearity of the method. This analytical evaluation used 7.5mL aliquots of blood collected from healthy women that were spiked with known numbers of live cultured tumor cells (between 0 - ~100 SKBR3, MCF7 or Hs 578T cells). The spiked blood samples were processed on Parsortix PC1 systems and the captured cells were harvested and deposited onto charged glass cytology slides (Cytospin slides) using the cytocentrifugation method. The cells on the slides were fixed, Wright-Giemsa stained, and identified using light microscopy. The results showed that significant cell loss occurred for all three of the cell lines evaluated as a result of the cytocentrifugation method used for the preparation of the cytology slides and/or the Wright-Giemsa staining procedure (Figure 8). The average percentages of SKBR3, MCF7 and Hs 578T cells observed on the Wright-Giemsa stained cytology slides over all of the spiking levels were ~31% (median = 28%), ~36% (median = 25%) and ~28% (median = 23%), respectively, indicating that 37% - 51% of the cells harvested by the Parsortix PC1 system were lost due to the cytology slide preparation method and/or Wright-Giemsa staining procedure (compared to harvesting the cells directly into 96-well plates).

![Figure 8. Comparison of linearity using live, SKBR3, MCF7 and Hs 578T cells spiked into 7.5mL of blood over a range of 2 to ~100 cells and processed using Parsortix PC1 Systems where captured cells were harvested and deposited onto Cytospin slides for identification using Wright-Giemsa staining.](image-url)

For the cytological evaluation of the HV subjects and MBC patients enrolled into the ANG-002 study, the cells harvested from the blood samples processed for the cytological evaluation were deposited onto charged glass cytology slides (Cytospin slides) using the cytocentrifugation method evaluated above. The
cells on the slides were fixed, Wright-Giemsa stained and reviewed by a single pathologist using light microscopy. Because cell identification by morphological analysis in the absence of specific markers is subjective, the following strict criteria for classifying cells were applied by the pathologist. Morphological features were used to classify cells as atypical (large cells with round nuclear contours and scant cytoplasm and/or cells with a hyperchromatic nucleus, often with irregular contours and scant/stripped cytoplasm), cells of unknown origin (cells with non-malignant features that did not represent hematopoietic precursors), naked nuclei (intact nuclei that exhibit no cytoplasm or minimally discernible cytoplasm), or cells with morphologic features of malignant cells (CTCs).

For all evaluable subjects [202 MBC patients and 192 HVs], the total number of CTCs and other non-normal cells observed in each sample (i.e., atypical cells, cells of unknown origin, and naked nuclei) were determined and summarized for the MBC patients and HV subjects. 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 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. In MBC patients, 15.8% had one or more cells classified as a malignant CTCs compared to 1.6% of the healthy volunteers. Similarly, the percentage of MBC patients with observed cells classified as non-normal (i.e. malignant CTCs, atypical cells, cells of unknown origin, and/or naked nuclei) (45.0%) was higher than that observed in healthy volunteers (18.2%). These results also showed that MBC patients with recurring/progressive metastatic disease (compared to those with newly diagnosed metastatic disease) were found to have one or more CTCs or other non-normal cells observed on their Wright-Giemsa stained cytology slides (20.9% and 6.8%, respectively).

Molecular evaluation of Parsortix PC1 cell Harvests: Material harvested by the Parsortix PC1 device from the evaluable HV subjects and MBC patients was subjected to several standard 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 that the harvested cells can be analyzed with a number of methods used to evaluate the molecular signature of circulating tumor cells that may be 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.

15. Troubleshooting

Potential problems and recommended actions related to Parsortix PC1 instrument operation are described in Section 11 of the PC1-OM-F Parsortix PC1 Instrument Instructions for Use. Contact ANGLE Technical Support if further advice is required (see Section 17 below).

16. Symbols Used

The Parsortix PC1 MBC-01 Metastatic Breast Cancer Kit carries the following hazard, warning and operational labels:

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>MEANING</th>
</tr>
</thead>
<tbody>
<tr>
<td>📚</td>
<td>READ INSTRUCTIONS</td>
</tr>
<tr>
<td>📚</td>
<td>Read the instruction manual for a description of principles of operation and details of potential hazards</td>
</tr>
</tbody>
</table>

Date: 09 January 2023
| **CE MARK** | Indicates compliance with a range of European Directives as set out in an accompanying EU Certificate of Conformity |
| **MANUFACTURER** | Name and address of the manufacturer |
| **EXPIRATION DATE USE BY YYYY-MM** | Indicates the expiration date, use by year and month |
| **CAUTION** | Consult the instructions for use for important warning or cautionary information |
| **LOT NUMBER** | Manufacturer’s lot number for traceability of components |
| **SINGLE USE ONLY** | The components must not be used more than once to avoid the risk of cross-contamination |
| **DO NOT USE IF PACKAGING IS DAMAGED** | Do not use the labeled component if the packaging is already open in order to avoid the risk of contamination |
| **KEEP AWAY FROM SUNLIGHT** | Parts labeled are sensitive to sunlight and should be stored away from sunlight |
| **FRAGILE** | Indicates that the contents of a package or container are fragile and should be handled with care |
| **CONTENTS SUFFICIENT FOR N TESTS** | Indicates the number (N as displayed) of tests (or related consumable items) supplied |
| **IN VITRO DIAGNOSTIC DEVICE** | The device is designated as an In Vitro Diagnostic Device and is for in vitro diagnostic use in accordance with the intended use statement |
| **Rx ONLY** | Prescription only – Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices to use, or order the use of, the device. |
| **CATALOG NUMBER** | Indicates the manufacturer’s catalogue number of the device to aid identification. |
| **EC REP** | Indicates the European Union Authorized Representative for the Parsortix system. |
17. Technical Support

Contact ANGLE Technical Support if further advice is required.

Authorized manufacturer’s representative in North America:

ANGLE North America Inc
5100 Campus Drive
Suite 120
Plymouth Meeting, PA 19462
Email: us-support@angleplc.com
www.angleplc.com

Device manufacturer:

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