**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**

**A. 510(k) Number:**
K050245

**B. Purpose for Submission:**
Reagent formulation changes and changes in procedural incubation times to deliver the same assay performance characteristics on the new automated version of the CellPrep semi-automated cell preparation instrument, now called CellSearch AutoPrep. Larger volumes of reagents are needed for automated use.

**C. Measurand:**
EpCam, Cytokeratins 8, 18 and/or 19, and CD45 to identify Circulating Tumor Cells (CTC) (Cells appearing to look like tumor cells with epithelial cell markers and no lymphocyte marker on their surfaces).

**D. Type of Test:**
A semi-automated qualitative immunomagnetic-capture immunofluorescent detection image analysis test.

**E. Applicant:**
Veridex, LLC, A Johnson and Johnson Company

**F. Proprietary and Established Names:**
CellSearch™ Circulating Tumor Cell Kit

**G. Regulatory Information:**

1. Regulation section:
   21 CFR 866.6020-Immunomagnetic Circulating Cancer Cell Selection and Enumeration System
2. Classification:
   Class II
3. Product code:
   NQI System, Immunomagnetic, Circulating Cancer Cell, Enumeration
4. Panel:
   Immunology 82

**H. Intended Use:**

1. Intended use(s):
   The CellSearch™ Circulating Tumor Cell Kit is intended for the enumeration of circulating tumor cells (CTC) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood.
2. Indication(s) for use:
   The presence of CTC in the peripheral blood, as detected by the CellSearch™ Circulating Tumor Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast cancer. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival.
3. Special conditions for use statement(s):
   For prescription use only.
4. Special instrument requirements:
   The CellTracks® AutoPrep system and the CellTracks® Analyzer II. The

CellTracks® Analyzer II is a semi-automated fluorescence microscope intended to enumerate fluorescently labeled cells that are immunomagnetically selected and distributed over a viewing surface

**I. Device Description:**

The CellSearch™ Circulating Tumor Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of nanoparticles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the EpCAM antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CTC. The fluorescent reagents include the following: anti-CK-Phycoerythrin (PE) specific for the intracellular protein cytokeratin (characteristic of epithelial cells), DAPI which stains the cell nucleus, and anti-CD45-Allophycocyanin (APC) specific for leukocytes

The reagent/sample mixture is dispensed by the CellTracks® AutoPrep System into a cartridge that is inserted into a MagNest® cell presentation device. The strong magnetic field of the MagNest® device attracts the magnetically labeled epithelial cells to the surface of the cartridge. The CellTracks® Analyzer II or CellSpotter® Analyzer automatically scans the entire surface of the cartridge, acquires images and displays any event to the user where CK-PE and DAPI fluorescence are co-located. Images are presented to the user in a gallery format for final classification. An event is classified as a tumor cell when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype EpCAM+, CK+, DAPI+ and CD45-

**J. Substantial Equivalence Information:**

1. Predicate device name(s):
   CellSearch™ Epithelial Cell Kit
2. Predicate 510(k) number(s):
   K031588
3. Comparison with predicate of modifications made to reagents:

| **Similarities** | | |
|---|---|---|
| Item | Predicate | Device |
| **Summary of changes** | CellSearch™ Epithelial Cell Kit | CellSearch™ Circulating Tumor Cell Kit |
| **Cell Fixative** There is no change to the Cell Fixative reagent between systems | • **2.5 mL bottle Cell Fixative:** Contains PBS, 25% proprietary ingredients, BSA and sodium azide. This bottle is color-coded with a **red** cap. | **3.0 mL bottle Cell Fixative:** Contains PBS, 25% proprietary ingredients, 0.1% BSA and 0.1% sodium azide. **(red cap)** |
| **Differences** | | |
| Item | Predicate | Device |
| **Anti-EpCAM Ferrofluid**No changes were made to theanti-Ep-CAM antibody used on the ferrofluid. Neither | **1.1 mL vial anti-EpCAM-Ferrofluid**: Contains a suspension of 0.03% magnetic nanoparticles conjugated to a mouse monoclonal antibody that is | **3.0 mL bottle Anti-EpCAM Ferrofluid**: Contains a suspension of 0.022% magnetic nanoparticles conjugated to a mouse |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device |
| were any changes made to the formulation of or manufacture of Anti-Ep-CAM ferrofluid; except for the change in final concentration required to accommodate the CellTracks® AutoPrep System required differences in volume of reagent added (150 μL versus 100 μL) and the slightly shorter incubation time | specific for a cell surface marker present on epithelial cells in a buffer containing bovine serum albumin (BSA) and ProClin 300 preservative. | monoclonal antibody specific for the cell surface marker EpCAM present on epithelial cells in a buffer containing 0.03% bovine serum albumin (BSA) and 0.05% ProClin 300 preservative. **(brown** cap) |
| **Nucleic Acid Dye**DAPI is the same dye for both systems. A modification was made in the formulation of the Nucleic Acid Dye in order to accommodate the CellTracks® AutoPrep System/ CellTracks® Analyzer II. The modification was to formulate in Normal Saline for AutoPrep instead of distilled water. This was done because adding 20 μL of distilled water to 260 μL of PBS-BSA, as was done in CellPrep, would not change the ionic strength of the media very much. In AutoPrep however, adding 150 μL of water to 700 μL of PBS-BSA would have had a large effect on the ionic strength. Therefore, the DAPI was formulated in normal saline. Cap color change | **0.25 mL vial Nucleic Acid Dye**: Contains 0.005% 4', 6-diamidino-2-phenylindole, dihydrochloride (DAPI) and ProClin 300. This vial is color-coded with a **purple** inset in the cap. | **3.0 mL bottle Nucleic Acid Dye**: Contains 0.005% 4', 6-diamidino-2-phenylindole, dihydrochloride (DAPI) and ProClin 300. **(blue** cap) |
| **anti-CK-PE and anti-CD45-APC**The original 3 Staining reagents have been combined into one Staining reagent. Same materials and small optimization change in monoclonal antibody concentration. The | **0.25 mL vial anti-CK-PE A**: Contains 0.001% mouse monoclonal antibodies specific to cytokeratins 8 and 18 conjugated to phycoerythrin (PE) in phosphate buffered saline (PBS) containing BSA, surfactant and sodium azide. This vial is color-coded with a **yellow** inset in the | **3.0 mL bottle Staining Reagent**: Contains 0.0008% mouse monoclonal antibodies specific to cytokeratins conjugated to phycoerythrin (PE); 0.0012% mouse anti-CD45 mouse monoclonal antibody conjugated to allophycocyanin (APC) in |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device |
| Staining reagent contains three fluorescent-probe-conjugated antibodies. The three conjugated antibodies are C11-PE, CK19-PE, and anti-CD45-APC. In AutoPrep, the exact same antibodies are used as are used for CellPrep. Each antibody is purified using the same procedures, conjugated to the same fluorochrome in the same 1:1 F/P ratio, using the same conjugation chemistry and procedures, and formulated in the same diluent as was used for the original CellSearch-CellPrep reagents. In CellSearch-CellPrep each antibody was formulated into the same dilution buffer, but was supplied as three separate reagents, each of which had to be added to the test sample in 20 μL aliquots. For AutoPrep, all three antibodies are instead formulated into one bottle so that addition of a single 150 μL of antibody staining reagent delivers all three antibodies. The concentrations of the antibodies as delivered to the test sample are slightly lower than was used in CellSearch-CellPrep. The final combination of antibodies was formulated to provide a robust mixture of antibody conjugates that would provide performance comparable to that of the individually formulated CellSearch-CellPrep reagents. Cap color is now white | cap.<br><br>**0.25 mL vial anti-CK-PE B**: Contains 0.001% mouse monoclonal antibodies specific to cytokeratin 19 conjugated to PE in PBS containing BSA, surfactant and sodium azide. This vial is color-coded with a **blue** inset in the cap.<br><br>**0.25 mL vial anti-CD45-APC**: Contains 0.005% mouse monoclonal antibodies specific to CD45 conjugated to allophycocyanin (APC) in PBS containing BSA, surfactant and sodium azide. This vial is color-coded with a **red** inset in the cap. | phosphate buffered saline (PBS) containing 0.5% BSA, and 0.1% sodium azide. (**white** cap) |
| **Aggregation agent in** | **60 mL bottle AB Dilution** | • **3.0 mL bottle Capture** |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device |
| **Buffer** No material changes.<br>The active ingredient of this reagent is Streptavidin. The Streptavidin reacts with desthiobiotin on the surface of the Ferrofluid particles to initiate the proprietary controlled aggregation technology. Streptavidin is present in great excess. For CellPrep, this aggregation reagent was formulated in dilution buffer and 6 mLs was added to bring the total volume of the blood sample to approximately 10 mLs. In AutoPrep, the aggregation reagent is formulated in PBS-BSA and the same amount (in µg) of Streptavidin is added (150 µL) to the 10 mL blood sample that already contains dilution buffer. | **Buffer**: Contains phosphate buffered saline (PBS), BSA, 0.6% other animal protein, 0.006% proprietary aggregation agent and sodium azide. | **Enhancement Reagent:** Contains PBS, 0.5% BSA, 0.02% proprietary reagent for controlled ferrofluid aggregation and 0.1% sodium azide. (**clear cap**) |
| **Permeabilization Reagent** The matrix used and the active ingredient of Permeabilization Reagent is the same for both CellPrep and AutoPrep. Due to the longer incubation time used on AutoPrep the concentration of Saponin is slightly lower than was used in CellPrep. In addition, for AutoPrep biotin was added to Permeabilization Reagent. Biotin is inert with respect to permeabilization and is also used in the CellSearch-CellPrep assay. The same component, biotin, is used in both CellPrep and AutoPrep to affect the deaggregation, and the action and mechanism | • **2.5 mL bottle Permeabilization Reagent**: Contains phosphate buffered saline (PBS), 0.05% proprietary permeabilization reagent and sodium azide. This bottle is color-coded with a **green** cap. | • **3.0 mL bottle Permeabilization Reagent**: Contains phosphate buffered saline (PBS), 0.011% proprietary permeabilization reagent and 0.1% sodium azide (**green cap)** |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device |
| remains unchanged between CellSearch-CellPrep and CellSearch-AutoPrep reagents. | | |

**K. Standard/Guidance Document Referenced (if applicable):**

The CellSearch™ Circulating Tumor Cell kit was developed in conformance to the following standards and guidances.

ISO 14971 Medical Devices- Application of Risk Management to Medical Devices

Guidance for Industry and FDA Staff Class II Special Controls Guidance Document: Circulating Cancer Cell Selection and Enumeration System (May 11,2004)

Guidance for Industry and FDA Staff: Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use (November 30, 2004)

The CellTracks Analyzer II performance data were developed in conformance to the following standards.

EP5-A NCCLS document: Evaluation of Precision Performance of Clinical Chemistry Devices

EP9-A NCCLS document: Method comparison and Bias Estimation Using Patient Samples

All requirements for these standards were met. EP9-A testing was performed using donor spiked samples rather than actual cancer patient samples.

**L. Test Principle:**

Epithelial cells are immunomagnetically labeled by targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen. Anti-EpCAM monoclonal antibodies conjugated to ferrofluid particles are colloidal and, when mixed with a sample containing the target epithelial cells, bind to the EpCAM antigen associated with the epithelial cells. After immunomagnetic selection of epithelial cells from 7.5 mL of blood, fluorescent reagents are added at this time to discriminate between the immunomagnetically selected cells. Anti-Cytokeratin – Phycoerythrin (CK-PE) stains the intracellular cytoskeleton cytokeratin proteins expressed in cells of epithelial origin, anti-CD45-Allophycocyan (CD45-APC) stains leukocytes and DAPI stains DNA present in the cell nucleus.

The processed reagent/sample mixture is dispensed by the CellTracks® AutoPrep System into a cartridge that is inserted into a MagNest® cell presentation device. The strong magnetic field of the MagNest® device causes the magnetically-labeled target cells to move to the surface of the cartridge. The cartridge is then placed on the CellTracks® Analyzer II for data acquisition and analysis. The CellTracks Analyzer II scans the entire surface of the cartridge with a series of fluorescence filters that are defined for a given assay and acquires images of PE, APC and DAPI fluorescence staining of the entire viewing surface.

After data acquisition is completed, the images are analyzed for any event where cytokeratin-PE and DAPI are within a specified space in the cartridge, i.e. indicating the possible presence of a cell with a nucleus that expresses cytokeratin. Images from

each fluorescent color as well as a composite image of the cytokeratin staining (green) and the nuclear staining (purple) are presented to the user in a gallery for final cell classification. A cell is classified as a tumor cell when it its EpCAM+ (i.e., it is captured), CK+, DAPI+ and CD45-. A check mark placed by the operator next to the composite images classifies the event as a Circulating Tumor Cell (CTC) and the software tallies all the checked boxes to obtain the CTC count.

The sponsor's data demonstrate that metastatic breast cancer patients with 5 or more CTC/per 7.5 mL of blood have a significantly greater probability for shorter progression free and overall survival than patients who have fewer than 5 CTC per 7.5 mL of blood.

This test methodology is new. It is hoped that further clinical studies reported in the scientific literature will corroborate the clinical study performed by the sponsor for submission to the FDA for the predicate device.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

(1) System Reproducibility with CellSearch™ Circulating Tumor Cell Control

Three separate CellSearch™ Circulating Tumor Cell Control samples were prepared and processed each day for over 30 days, per the long run method of NCCLS guideline EP5-$A^2$. Each single-use sample bottle contains a low and a high concentration of cells from a fixed cell line that have been pre-stained with two different fluorochromes. Summary statistics for the high and low control cells is presented below.

**Table 1. Summary of Precision Analyses**

| | *Low* | **High** |
|---|---|---|
| N | 99 | 99 |
| Mean cell count | 48 | 969 |
| Total Precision Standard Deviation ($S_T$) % CV | 18% | 5% |

(2) System Reproducibility with Patient Samples

A total of 163 duplicate samples were collected from 47 patients over the course of the clinical study. These samples were processed at multiple sites to determine the reproducibility of CTC measurements. The regression equation for the comparison of these 163 duplicate samples was $Y=0.98x + 0.67$, $R^2=0.99$. **Table 2** shows the summary of the data for replicates where the average of the two CTC results was <5 compared to those where the average (avg.) was ≥5.

**Table 2. Reproducibility of CTC Counts in Duplicate Samples (n=163) with an Average of <5 or ≥5 CTC per 7.5 mL of blood.**

| | **CTC <5** | **CTC ≥5** |
|---|---|---|
| **Number of Duplicates** | 123 | 40 |
| **Mean CTC Count of Duplicates** | 0.7 | 210 |
| **Avg. Duplicate Standard Deviation** | 0.5 | 12 |
| **Avg. %CV of Duplicates** | 60% | 20% |

*Accuracy/Recovery*:

Blood samples from a single healthy donor were pooled and five of six 7.5 mL aliquots were spiked with 5, 20, 81, 325 and 1300 cultured breast cancer cells (SK-Br-3). The sixth tube was unspiked pooled blood and served as a zero point. These samples were processed on the CellTracks® AutoPrep System with the CellSearch™ Circulating Tumor Cell Kit and CTC counts were determined on the CellTracks® Analyzer II. The experiment was repeated for four additional donors. The observed cell counts were plotted against the results of the expected cell count. The results are summarized in **Table 3**.

**Table 3. Percent Detection Estimates.**

| Expected Tumor Cell Count | Mean Observed Tumor Cell Count | Range of Percent Recovery |
|---|---|---|
| 1300 | 1215 | 91 to 95% |
| 325 | 308 | 82 to 101% |
| 81 | 85 | 80 to 136% |
| 20 | 22 | 95 to 140% |
| 5 | 7 | 120 to 200% |

To determine the overall, or least squares fit, for the comparison of the observed and expected cell counts across all the data, linear regression analysis was performed. The regression equation for these 30 samples was $Y=0.93x + 3.87$, $R^2=0.999$. The results of this study indicate that on average over the tested CTC range the recovery, as derived from regression analysis, is 93%.

Given the linear response of the tumor cell counts, one would expect the slope of the observed versus expected plot to be 1.0. However, the slope was 0.93. This is because the CellTracks® AutoPrep System with CellSearch™ CTC Kit involves the capture and fluorescent labeling of cells followed by their detection and enumeration by the CellTracks® Analyzer II. The loss of cells could therefore be attributed to one of the following possibilities; 1) the recovery of only 93% of the tumor cells spiked into 7.5mL of blood by the CellTracks® AutoPrep System, 2) the detection of only 93% of the tumor cells present in the sample chamber by the CellTracks® Analyzer II or 3) a combination of both of these sources of error.

*b. Linearity/assay reportable range:*

Another way to examine the previous data is to analyze it as a dilution series to evaluate test linearity. We removed the confounding variable of percent recovery by using the observed value of the original sample divided by the dilution factors to determine the expected values for the dilution series for each patient sample. Regression of all of these numbers of observed tumor

cells versus the numbers of expected tumor cells yielded a slope of 1.007, an intercept of 3.0, an $r^2 = 0.99$ and $r = 0.995$. Therefore, once the percent recovery (cell loss) was factored out of the CTC values of each of the original samples, this analysis of the data demonstrated that the detection of CTC was linear over the reportable range of 0 to 1238 tumor cells.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No recognized reference material or method.

d. *Detection limit:*
One CTC per 7.5 mL can be detected by the CellTracks® Analyzer II resulting in a limit of detection of 1 CTC in a cartridge. Linear regression shows that on average, 93% of CTC present in a 7.5 mL blood sample are recovered using the CellTracks® AutoPrep System (see **Recovery** section). The loss of approximately 7% of the CTC in the sample is not sufficient to reduce the limit of detection of 1 CTC.

e. *Analytical specificity:*
The CellSearch™ Circulating Tumor Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of nanoparticles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the EpCAM antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CTC. The fluorescent reagents include the following: anti-CK-Phycoerythrin (PE) specific for the intracellular protein cytokeratin (characteristic of epithelial cells), DAPI which stains the cell nucleus, and anti-CD45-Allophycocyanin (APC) specific for leukocytes

*Interfering Substances*:

SK-BR-3 cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls. Toxic levels (5 times therapeutic index) of the following cancer drugs, over-the-counter drugs, and other exogenous substances were tested: cyclophosphamide, Mitomycin C®, Procrit®, biotin, 5-fluorouracil, methotrexate, tamoxifen citrate, paclitaxel, Arimidex®, acetaminophen, acetylsalicylic acid, caffeine, dextromethorphan, Aredia®, Human Anti-Mouse Antibody (HAMA) type 1, HAMA type 2, Herceptin®, and ibuprofen. No significant differences in SK-BR-3 cell numbers were detected, indicating that these substances do not interfere with the CellSearch™ kit.

Samples spiked with toxic levels of doxorubicin resulted in aberrant staining of leukocytes as cytokeratin and CD45 dual positive cells, due to the doxorubicin being a fluorescent compound that is incorporated into nucleated cells. If seen, the staining pattern of all cells being CD45 positive and cytokeratin positive is obvious and easily identified by the operator as a known interference staining profile. If blood is drawn after the recommended 7-day washout period, following doxorubicin infusion, this interference is unlikely to be observed in clinical practice given controlled therapeutic levels and rapid drug clearance.

Potential interference from lipemia was studied by adding Intralipid to

samples to a concentration of 2.6%, which corresponds to greater than 1000 mg/dl triglyceride. Samples were lysed to simulate total hemolysis. Bilirubin at 7.4 mg/dL, HAMA 1/HAMA 2 and hematocrit from 18-60% were studied. Lipemia, hemolysis, icterus and a broad range of hematocrit values do not interfere with the CellSearch™ test. HAMA 1 and HAMA 2 also do not interfere, indicating that individuals receiving mouse Ig by parenteral routes can be tested successfully with the CellSearch™ test.

*f. Assay cut-off:*

Results are reported as the number of CTC / 7.5 mL of blood. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival. This cut-off was established in the 510(k) of the previous version of this assay, the predicate device, K031588.

2. Comparison studies:

*a. Method comparison with predicate device:*

To demonstrate comparable performance between the new assay and the predicate system, a study was performed using duplicate samples split between both the predicate device, CellSearch™ Epithelial Cell System (K031588), and the new device, CellSearch™ Circulating Tumor Cell System (K050245). The study consisted of spiking normal donor whole blood samples with three different tissue culture lines (SKBr-3, PC3-9 and MCF-7) at three different levels (~5, ~50, and ~1000) for 5 days. The three cell lines (SK-Br-3, MCF-7, or PC3-9) were chosen to cover a broad range of EpCAM and Cytokeratin antigen density representing the capture and detection portions of the assay respectively. Three spike levels of each cell line were chosen to cover a range of potential clinical values. Of the three cell lines tested, the PC3-9 cell line has the lowest Cytokeratin antigen density. SK-Br-3 cells demonstrate an uneven bimodal population consisting primarily of moderate level Cytokeratin antigen density cells and a smaller population of higher expressing cells. MCF-7 cells demonstrate the highest level of consistent Cytokeratin expression. The Cytokeratin antigen is the target of the detection reagent for tumor cells in the CellSearch™ Circulating Tumor Cell kits. The design and execution of this study is consistent with the NCCLS guideline EP9-A. A total of 45 samples were analyzed on each of the two platforms

For MCF-7 cells the slope of the regression line = 1.03, an intercept of 1.5 and an $r^2 = 0.994$. For SKBr-3 cells, the slope of the regression line = 1.01 with an intercept of 2.9 and an $r^2 = 0.984$. For PC3-9 cells, the slope of the regression line = 1.19 with an intercept of 10.5 and an $r^2 = 0.963$. Analysis of data from all 3 tumor cell lines combined shows a slope of the regression line = 1.09 with an intercept of 1.5 and an $r^2 = 0.966$.

The slope of 1.19 for PC3-9 cells may be due to an improved dynamic range of the AutoPrep / CellTracks Analyzer II system resulting in a flattening out of the response curve at higher cell numbers. In other words, the recovery of CTC by the AutoPrep / CellTracks Analyzer II platform at high numbers of cells may be somewhat more sensitive than recovery by the CellPrep / CellSpotter platform, particularly with lower EpCAM antigen density cells as

is the case with PC3-9 cells (Figure 1). This difference could also be attributable to increased reliability and/or stability of the AutoPrep as compared to the CellPrep for sample preparation. Regardless of this potential difference however, there appears to be no difference between the AutoPrep / CellTracks Analyzer II platform and the CellPrep / CellSpotter platform at the medical decision level of 5-50 CTC's.

*b. Matrix comparison:*
Since whole blood is the only matrix for this test., no matrix comparison studies were performed.

3. Clinical studies:
   *a. Clinical Sensitivity:*
   Not applicable
   *b. Clinical specificity:*
   Not applicable
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable

All of the above new analytical studies with the CellSearch™ Circulating Cell Kit/ AutoPrep/CellTracks® Analyzer II system demonstrate that the detection of tumor cells by the CellSearch™ Circulating Cell Kit/ AutoPrep/CellTracks® Analyzer II system is substantially equivalent to the predicate system. Therefore, the following clinical data generated using the predicate system (K035188) is applicable to the new device (K050245).

**Metastatic Breast Cancer Patients**
A multi-center prospective, longitudinal clinical trial was conducted. Results were used to determine whether the number of CTC predict disease progression and survival. Patients with measurable disease and who were starting a new line of therapy were enrolled (N=177). Clinical data were analyzed on an intent-to-treat basis.

**Table 4. Patient Demographics**

| | | |
|---|---|---|
| **Age at Baseline (Median)** | | 58.0 ± 13.4 (58) |
| **Race:** | **White** | 153 (84%) |
| | **Black** | 14 ( 8%) |
| | **Hispanic** | 7 ( 4%) |
| | **Unknown** | 3 ( 2%) |
| | **ER/PR +** | 121 (68%) |
| | **ER/PR -** | 54 (31%) |
| | **Unknown** | 2 ( 1%) |
| | **Her-2/neu -** | 91 (52%) |
| | **Her-2/neu 1+** | 12 ( 7%) |
| | **Her-2/neu 2+** | 18 (10%) |
| | **Her-2/neu 3+** | 27 (15%) |
| | **Unknown** | 29 (16%) |
| **Line of Therapy** | | |
| | 1st | 83 (47%) |
| | 2nd | 25 (14%) |
| | ≥ 3rd | 67 (38%) |
| | Unk.* | 2 ( 1%) |
| **Type of Therapy** | | |
| | Hormone | 47 (26%) |
| | Chemo | 87 (49%) |
| | Immu/C/H | 28 (16%) |
| | H / C | 10 ( 6%) |
| | No Tx** | 4 ( 2%) |
| | Unk.* | 1 ( 1%) |

*Unk. = Information not available **No Tx. = No treatment information obtained
C or Chemo = Chemotherapy, H or Hormone = Hormone Therapy, I or Immuno = Immunotherapy

Baseline CTC count was determined prior to initiation of a new line of therapy. A first follow-up CTC count was determined after the initiation of therapy. For the baseline analyses, Progression Free Survival (PFS) was measured from the time of the baseline blood draw to the diagnosis of progression by CT scans and/or clinical signs and symptoms, and Overall Survival (OS) was measured from the time of baseline blood draw to the time of death. For the first follow-up analyses, PFS was measured from the time of 1st follow-up blood draw (mean 4.5 ± 2.4 weeks following enrollment) to diagnosis of progression or death, and OS was measured from the time of 1st follow-up blood draw to the time of death.

**Progression Free Survival (PFS) Analysis**

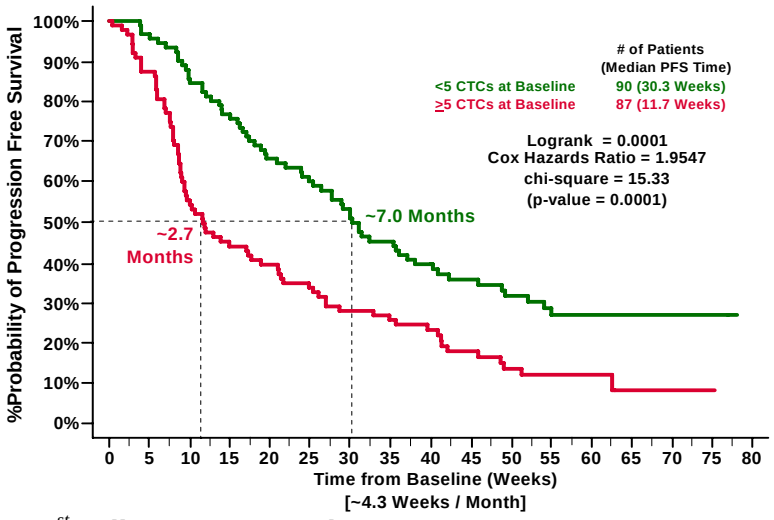
*PFS Using Baseline CTC Results*

All 177 patients had a baseline CTC test performed. For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at baseline:

- The Favorable group (N=90), represented in **green**, consisted of patients with <5 CTC.
- The Unfavorable group (N=87), represented in **red**, consisted of patients with ≥5 CTC.

Median PFS was 30.3 weeks (~7.0 months) for the Favorable group and 11.7 weeks (~2.7 months) for the Unfavorable group. The difference in PFS between the two groups is highly significant (Log-rank p=0.0001, Cox Hazards Ratio=1.9547, chi-square=15.33, p = 0.0001). These results are illustrated in **Figure 1**.

**Figure 1. PFS of Patients with < 5 or ≥ 5 CTC at Baseline (N=177)**



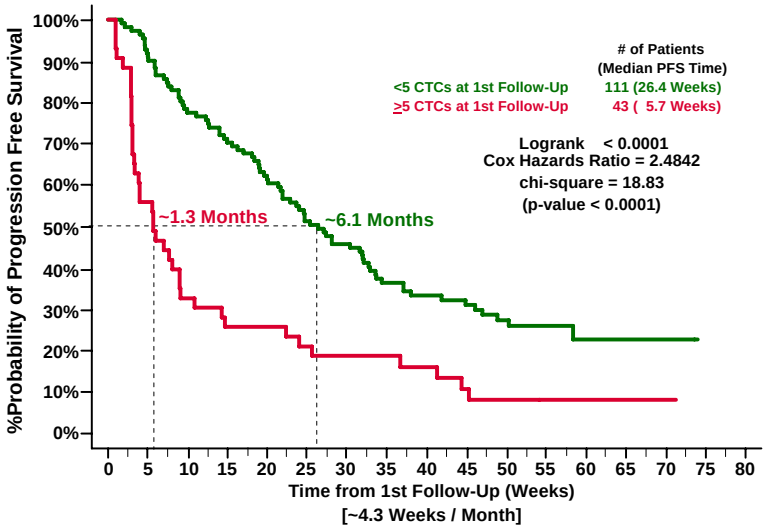

*PFS Using 1st Follow-up CTC Results*

Of the 177 patients, 23 were not evaluable at first follow-up. Of these 23 patients, ten patients died before a follow-up blood draw could be obtained, nine patients progressed prior to the 1st follow-up blood draw, and four were lost to follow-up. Additionally, the ten patients who died had high to extremely high CTC counts at baseline (CTC counts 9, 11, 15, 24, 111, 126, 301, 1143, 4648 and 23618). For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at 1st follow-up:

- The Favorable group (N=111), represented in **green**, consisted of patients with <5 CTC,
- The Unfavorable group (N=43), represented in **red**, consisted of patients with ≥5 CTC.

Median PFS was 26.4 weeks (~6.1 months) for the Favorable group and 5.7 weeks (~1.3 months) for the Unfavorable group. The difference in PFS between the two groups is highly significant (Log-rank p<0.0001, Cox Hazards Ratio=2.4842, chi-square=18.83, p< 0.0001). These results are illustrated in **Figure 2**.

**Figure 2. PFS of Patients with < 5 or ≥ 5 CTC at 1st Follow-Up (N=154)**




*Predictive Value of CTC Reduction on PFS*

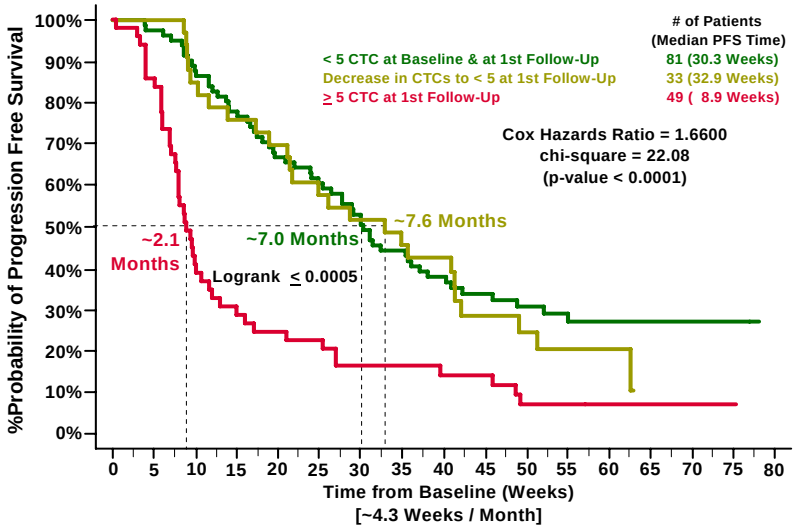
For Kaplan-Meier analysis, patients were segmented into three groups based on their

CTC counts at baseline and 1st follow-up:

- The Favorable group (N=81), represented in **green**, consisted of patients with <5 CTC at both time points,
- Patients with 5 or more CTC at baseline that decreased to below 5 CTC at 1st follow-up are represented in **olive green** (N=33),
- The Unfavorable group (N=49), represented in **red**, consisted of patients with ≥5 CTCs at 1st follow-up,

Elapsed PFS time was calculated from the baseline blood draw. Three groups were plotted in **Figure 3**. The Favorable group (N=81, **green** line) had a median PFS of 30.3 weeks (~7.0 months) and the patients represented by the **olive green** line (N=33) had a median PFS of 32.9 weeks (~7.6 months). The Unfavorable group (N=49, **red** line) had a median PFS of 8.9 weeks (~2.1 months). The difference in the PFS of the patients in the Favorable and **olive green** groups compared to the PFS of the patients in the Unfavorable group is highly significant (Log-rank p≤0.0006, Cox Hazards Ratio=1.6600, chi-square=22.08, p< 0.0001).

**Figure 3. A Reduction in CTC Count to Below 5 at the 1st Follow-Up Time Point After the Initiation of Therapy Predicts Improved PFS (N=163)**



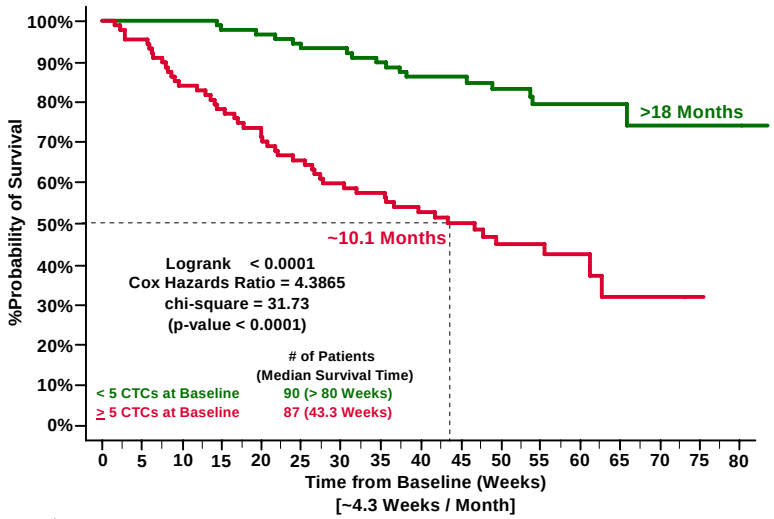

**Overall Survival (OS) Analysis**

*OS Analysis Using Baseline CTC Results*

Death occurred in 66 (37%) of the 177 patients during this study. Seventeen (19%) of 90 patients from Favorable group (<5 CTC at baseline) compared to 49 (56%) of 87 from Unfavorable group (≥5 CTC at baseline) died. Median OS was greater than 80 weeks (>18 months) for the Favorable group and 43.3 weeks (~10.1 months) for the Unfavorable group. The OS difference between the two groups is highly significant (Log-rank p<0.0001, Cox Hazards Ratio=4.3865, chi-square=31.73, p< 0.0001). These results are illustrated in **Figure 4**. For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at baseline:

- The Favorable group (N=90), represented in **green**, consisted of patients with <5 CTC.
- The Unfavorable group (N=87), represented in **red**, consisted of patients with ≥5 CTC.

**Figure 4. OS of Patients with < 5 or ≥ 5 CTC at Baseline (N=177)**



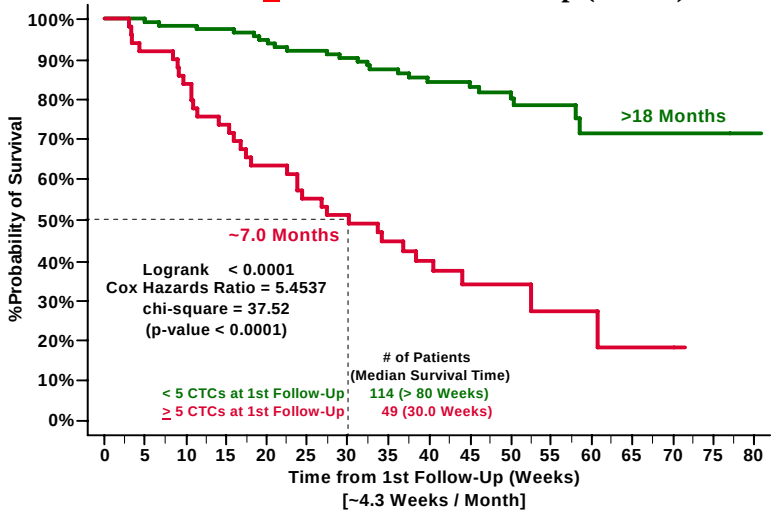

*OS Using 1st Follow-up CTC Results*

For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at 1st follow-up:

- The Favorable group (N=114), represented in **green**, consisted of patients with <5 CTC,
- The Unfavorable group (N=49), represented in **red**, consisted of patients with ≥5 CTC.

Of the 163 evaluable patients at first follow-up, 56 (34%) died during this study; 23 of 114 (20%) from the Favorable group, 33 of the 49 (67%) from the Unfavorable group. Patients in the Favorable group had a median survival of greater than 80 weeks (>18 months), while the Unfavorable group had a median OS of 30 weeks (~ 7.0 months). The difference in OS between the two groups is highly significant (Log-rank p<0.0001, Cox Hazards Ratio=5.4537, chi-square=37.52, p< 0.0001). Results are summarized in **Figure 5**.

**Figure 5. OS of Patients with < 5 or ≥ 5 CTC at 1st Follow-Up (N=163)**




*Predictive Value of CTC Reduction on OS*

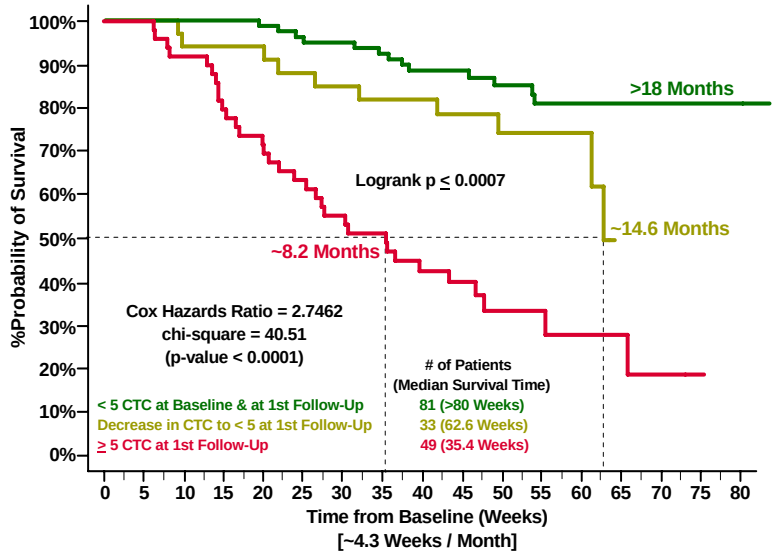
For Kaplan-Meier analysis, patients were segmented into three groups based upon their CTC counts at baseline and 1st follow-up:

- The Favorable group (N=81), represented in **green**, consisted of patients with <5 CTC at both time points,

- Patients with 5 or more CTC at baseline that decreased below 5 CTC at 1st follow-up are represented by the **olive green** line (N=33),
- The Unfavorable group (N=49), represented in **red**, consisted of patients with ≥5 CTC at 1st follow-up,

Elapsed OS time was calculated from the baseline blood draw. **Figure 6** illustrates that a decrease to <5 CTC after the initiation of therapy significantly impacts OS. The Favorable group (**green** line) had a median OS of >80 weeks (18 months). The patients represented by the **olive green** line (N=33) had a median OS of 62.6 weeks (~14.6 months). The Unfavorable group (**red** line) had a median OS of 35.4 weeks (~8.2 months). This difference in the OS of the patients in the Favorable and **olive green** groups compared to the OS of the patients in the Unfavorable group is highly significant (Log-rank p≤0.0007, Cox Hazards Ratio=2.7462, chi-square=40.51, p<0.0001). These data suggest that baseline and 1st follow-up CTC levels are predictive of overall survival.

**Figure 6. A Reduction in CTC Count to Below 5 at the 1st Follow-Up Time Point After the Initiation of Therapy Predicts Improved OS (N=163)**




**Multivariate Cox Regression Analysis**

The following parameters were evaluated using multivariate Cox regression analysis, with the SAS PROC PHREG (regression Analysis of Survival Data Based on the Cox Proportional Hazards Model), stepwise selection process to evaluate association with PFS and OS: patient age (continuous), stage of disease at diagnosis (I-IV), time to metastasis (continuous), ECOG status before initiation of a new line of therapy (0-2), ER/PR status (+/-), HER2/neu status (0-3), line of therapy (≥2nd or 1st), type of therapy (chemo/other or hormonal/immuno), baseline CTC count (≥5 or <5 CTC/7.5mL), and 1st follow-up CTC count (≥5 or <5 CTC/7.5mL). A stringency level (p-value) of 0.05 was used to both include and exclude parameters in the multivariate analyses. Results for each parameter that demonstrated a statistically significant correlation to PFS and OS are summarized in **Tables 5 and 6**, respectively. CTC number was the strongest predictor of PFS and OS.

**Table 5. Multivariate Cox Analysis: Stepwise Cox Regression for Prediction of PFS**

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Unfavorable | Favorable | HR | p-value | $chi^2$ | # of Patients |
| Baseline CTC Number | ≥5 | <5 | 1.761 | 0.001 | 10.58 | 172 |
| Line of Therapy | ≥2nd | 1st | 1.725 | 0.002 | 9.75 | |
| Type of Therapy | Chemo/Other | Hormonal/Immuno | 1.611 | 0.016 | 5.85 | |

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Unfavorable | Favorable | HR | p-value | $chi^2$ | # of Patients |
| 1st Follow-Up CTC Number | ≥5 | <5 | 2.516 | < 0.001 | 23.56 | 162 |
| Line of Therapy | ≥2nd | 1st | 1.579 | 0.013 | 6.22 | |

**Table 6. Multivariate Cox Analysis: Stepwise Cox Regression for Prediction of OS**

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Unfavorable | Favorable | HR | p-value | $chi^2$ | # of Patients |
| Baseline CTC Number | ≥5 | <5 | 4.261 | < 0.001 | 22.35 | 170 |
| Line of Therapy | ≥2nd | 1st | 2.384 | 0.001 | 10.32 | |
| Type of Therapy | Chemo/Other | Hormonal/Immuno | 2.543 | 0.015 | 5.90 | |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.478 | 0.024 | 5.10 | |
| Time to Metastasis | Time in Years | | 0.922 | 0.028 | 4.82 | |

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Unfavorable | Favorable | HR | p-value | $chi^2$ | # of Patients |
| 1st Follow-Up CTC Number | ≥5 | <5 | 6.493 | < 0.001 | 38.34 | 160 |
| ER/PR Status | Positive | Negative | 0.349 | 0.001 | 11.19 | |
| Line of Therapy | ≥2nd | 1st | 2.291 | 0.006 | 7.67 | |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.530 | 0.025 | 5.05 | |

4. Clinical cut-off**:**
   Results are reported as the number of CTC / 7.5 mL of blood. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival. This cut-off was established in the 510(k) of the previous version of this assay, the predicate device, K031588.
5. Expected values/Reference range:
   **EXPECTED VALUES**
   The EXPECTED VALUES were determined with the previous version of this test, the predicate device, K031588. The performance data demonstrates that the two products give similar results. Thus, the EXPECTED VALUES are applicable to both assay systems.

   *Healthy volunteers, non-malignant breast disease, non-malignant other disease*
   Single point CTC analyses were performed on control groups of 145 healthy volunteers, 101 women with non-malignant breast disease, and 99 women with other non-malignant diseases.

   Epithelial cells are not expected to be present in the peripheral blood of healthy individuals. Of the 345 total samples from healthy volunteers and women with non-malignant disease, only one subject had more than 5 CTC/7.5 mL. The results are presented in **Table 1**.

**Table 7. Control Subjects**

| Category | N | Mean # CTC | SD | # Patients with ≥ 5 CTC | Min.* | Max.* |
|---|---|---|---|---|---|---|
| **Healthy** | 145 | 0.1 | 0.2 | 0 | 0 | 1 |
| **Non-malignant breast disease** | 101 | 0.2 | 1.2 | 1 | 0 | 12 |
| **Non-malignant other disease** | 99 | 0.1 | 0.4 | 0 | 0 | 3 |

* NCCLS Guideline C28-A2[3]

## N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

## O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.