

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

A. 510(k) Number:

k031588

B. Analyte:

EpCam, Cytokeratins 8, 18 and/or 19, and CD45

C. Type of Test:

Semi-automated Immunomagnetic and immunofluorescent

D. Applicant:

Veridex, LLC, A Johnson and Johnson Company

E. Proprietary and Established Names:

Cell Search Epithelial Cell Kit and CellSpotter Analyzer

F. 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:
Pathology 88

G. Intended Use:

The CellSearch™ Epithelial 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.

1. Indication(s) for use:
The presence of CTC in the peripheral blood, as detected by the CellSearch™ Epithelial Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast cancer.
2. Special condition for use statement(s):
For prescription use only.
3. Special instrument Requirements:
The CellPrep™ Semi-Automated Cell Preparation System and the CellSpotter™ Analyzer. The CellSpotter™ Analyzer is a semi-automated fluorescence microscope intended to enumerate fluorescently labeled cells that are immunomagnetically selected and distributed over a viewing surface

H. Device Description:

The CellSearch™ Epithelial Cell Kit analyzed on the CellSpotter™ Analyzer is called the CellSearch Assay. The CellSearch Assay is a semi-automated *in vitro* diagnostic device.

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 cyto keratin proteins expressed in cells of epithelial origin, anti-CD45-Allophycocyan (CD45-APC) stains leukocytes and DAPI stains DNA present in the cell nucleus. A strong magnetic field is applied to the processed reagent/sample mixture that causes the labeled target cells to move to the cartridge surface. The cartridge is then placed on the CellSpotter™ Analyzer for data acquisition and analysis. The CellSpotter™ Analyzer 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 cyto keratin-PE and DAPI are within a specified space in the CellSpotter™ Cartridge, i.e. indicating the possible presence of a cell with a nucleus that expresses cyto keratin. Images from each fluorescent color as well as a composite image of the cyto keratin 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 is 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.

I. Substantial Equivalence Information:

1. Predicate device name(s):
None.
2. Predicate K number(s):
None.
3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Not Applicable	Not Applicable
B. Differences	
Not Applicable	Not Applicable

J. Standard/Guidance Document Referenced (if applicable):

1. NCCLS Approved Guideline EP5A, “Evaluation of Precision Performance of Clinical Chemistry Devices”.
2. NCCLS Approved Guideline C28-A2, “How to Define and Determine Reference Intervals in the Clinical Laboratory”.

K. Test Principle:

The CellSearch™ Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of nano-particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents are added for

identification and enumeration of CTC. Anti-CK-PE is specific for the intracellular protein cytokeratin (characteristic of epithelial cells), DAPI stains the cell nucleus, and anti-CD45-APC is specific for leukocytes.

The reagent/sample mixture is dispensed by the CellPrep™ Semi-Automated Cell Preparation System into a CellSpotter™ Cartridge that is inserted into a MagNest™ fixture, a device of two magnets held together by steel. The strong magnetic field of the MagNest™ fixture causes the magnetically-labeled epithelial cells to move to the surface of the cartridge. The CellSpotter™ Analyzer automatically scans the entire surface of the CellSpotter™ Cartridge, acquires images and displays any event to the user where CK-PE and DAPI are co-located. Images are presented to the user in a gallery format for final classification of the magnetically captured cells. An event is classified as a tumor cell when its morphological features are consistent with that of a cell and it exhibits the correct phenotype, i.e., EpCAM+, CK+, DAPI+ and CD45-.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

i. System Reproducibility with Spiked Specimens

Each day, blood from a single healthy donor was pooled and 7.5 mL samples were spiked with SKBr-3 cells. Low cell spikes (~58 cells/7.5mL) and high cell spikes (~319 cells/7.5mL) were prepared on CellPrep™ instruments and run in duplicate twice each day. This process was repeated each day for a period of 20 days as per NCCLS guideline EP5-A using blood from a total of twenty different normal donors. Over the course of the study, three operators, two different CellPrep™ Systems and two different CellSpotter™ Analyzers were used to generate the cell count data. Summary statistics for cell counts of the spiked samples are presented in **Table 1**.

Table 1. Summary of Precision Analyses

	Low	High
N	80	80
Mean CTC Count per 7.5mL	47	258
Total Precision Standard Deviation (S_T) % CV	15.8%	9.4%

ii. System Reproducibility with Patient Specimens

A total of 163 duplicate samples were collected from 47 patients over the course of the clinical study. These samples were processed separately on multiple systems at different sites (including different CellPrep™ instruments) to determine the reproducibility of CTC measurements. The regression equation for the comparison of these 163 duplicate samples was $y=0.98x + 0.67$, $r^2=0.9978$. **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 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	210.5
Avg. Duplicate Standard Deviation	0.5	12.0
Avg. %CV of Duplicates	60.0%	20.0%

b. Linearity/assay reportable range:

Blood from a single healthy donor was pooled and five 7.5 mL samples were spiked with SKBr-3 cells. Serial dilutions were performed resulting in twenty-five samples with expected cell counts ranging from 4 to 1022 cells per 7.5 mL. These samples were processed and analyzed over five consecutive days and the observed cell count results were plotted against the expected cell counts. 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 25 samples was $y=0.99x + 5.71$, $r^2=0.9912$. The 95% confidence interval of the intercept of 5.71 was -0.29 to 11.4, which overlaps zero. In this analytical study, the CellSearch™ Kit was shown to be linear over the tested range from 4 to 1022 cells.

c. Traceability (controls, calibrators, or method):

No recognized reference material or methods.

d. Detection limit (functional sensitivity):

One CTC per 7.5 mL of blood can be detected by the CellSpotter™ Analyzer resulting in a limit of detection of 1 CTC in a CellSpotter™ Cartridge. An average of 85% of CTC are recovered through the sample 7.5 mL processing (see **Recovery** section), the CTC loss of approximately 15% is not sufficient to reduce the analytical sensitivity of 1 CTC in a 7.5 mL blood sample. This CTC loss however does indicate that no CTC will be detected in approximately 15 of 100 samples in which only one EpCAM+, cytokeratins 8, 18, and/or 19+, CD45- circulating tumor cell is present in 7.5 mL of blood.

e. Analytical specificity:

Interfering Substances - SKBr-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 SKBr-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 fluorescence 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 outside of the recommended 7 day wash-out 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 is 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™ assay. 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™ assay.

f. Assay cut-off:

A CTC count of 5 or more per 7.5mL of blood is predictive of shorter progression free survival and overall survival.

Median progression free survival (PFS) times and Cox hazard's ratios over a range of circulating tumor cell (CTC) cutoffs in the Training Set using 1st follow-up data were used to determine the optimal CTC cutoff for the prediction of PFS. The PFS time for each patient used in this analysis was calculated from the date of the 1st follow-up. Ninety (90) of the 102 patients in the Training Set were not diagnosed with disease progression before or at first follow-up (**Table 2**). For every possible cutoff from 1 to 82 CTC, and at selected cutoffs up to 10,000 CTC, the median PFS was calculated for the positive and negative patient groups. In addition, the percentage of positive patients at each CTC cutoff was calculated. The following criteria, listed according to priority, were used to determine the optimal CTC cutoff:

1. The CTC cutoff must be above normal background (See Table 3)
2. The CTC cutoff at which the median PFS initially reaches a plateau in the positive patients (**red** line, **Figure 1**).
3. The CTC cutoff at or adjacent to the plateau defined in #2 above, which has the highest Cox hazard's ratio (**black** line, **Figure 1A**).

The determination of the CTC cutoff in the 90 patient Training Set is illustrated in **Figure 1**. In **Figure 1** the median PFS for positive patients (**red** line), negative patients (**green** line), and the Cox hazard ratio (**black** line) are plotted against the various CTC cutoffs. Only cutoffs with a value change are shown. For example, the CTC cutoffs of 16 to 19 are not shown on the graph because the median PFS of positive and negative patients and the Cox hazard's ratio at these cutoffs are the same as the cutoff of 15 CTC. **Figure 1** clearly demonstrates that the presence of CTC has important prognostic implications for the patients. The median PFS of all 90 patients is 5.0 months and is represented in the figure by the 0 CTC cutoff value (**blue** dot). As an example, at a CTC cutoff of ≥ 1 , patients with < 1 (**green** line) CTC had a median PFS of 6.3 months whereas patients with ≥ 1 CTC (**red** line) had a PSF time of 3.4 months.

The median PFS reached an initial plateau in the positive patient group at a PSF of 1.4 months for a cutoff of 5 or more CTC (criterion 2 above). This 5 CTC cutoff is well above the normal CTC background of 0 to 2 CTC (criterion 1 above). The third criterion used to define the cutoff was the Cox hazard's ratio; at 5 CTC the Cox hazard's ratio was 2.41, at 4 CTC it was 2.36 and at 6 CTC it also was 2.34. Thus, the 5 CTC cutoff provided the highest Cox hazard's ratio at or adjacent to the plateau defined by the median PFS. Based on the criteria outlined above, a cutoff of 5 or more CTC was chosen, and is indicated by a **yellow** line in **Figure 1**.

The cutoff of 5 CTC was determined using only results from the 1st follow-up and PFS as the outcome. Additional analyses have shown that the cutoff for OS at the 1st blood draw, as well as for PFS and OS at baseline, may differ from 5 CTC. For purposes of uniformity and simplicity in the interpretation of test results (by the clinician), a CTC cutoff of 5 was used for all analyses.

Figure 1. Determination of CTC Cutoff Using Median PFS in Training Set (n=90)

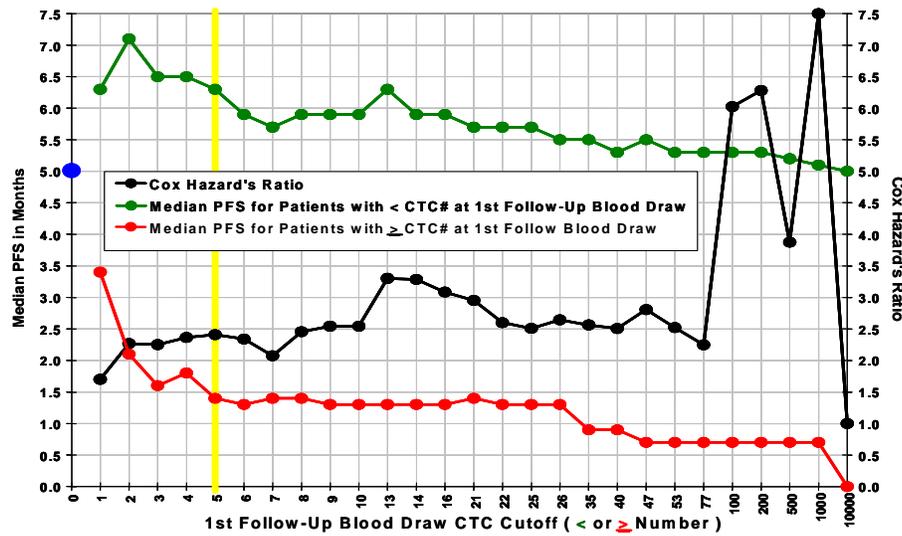
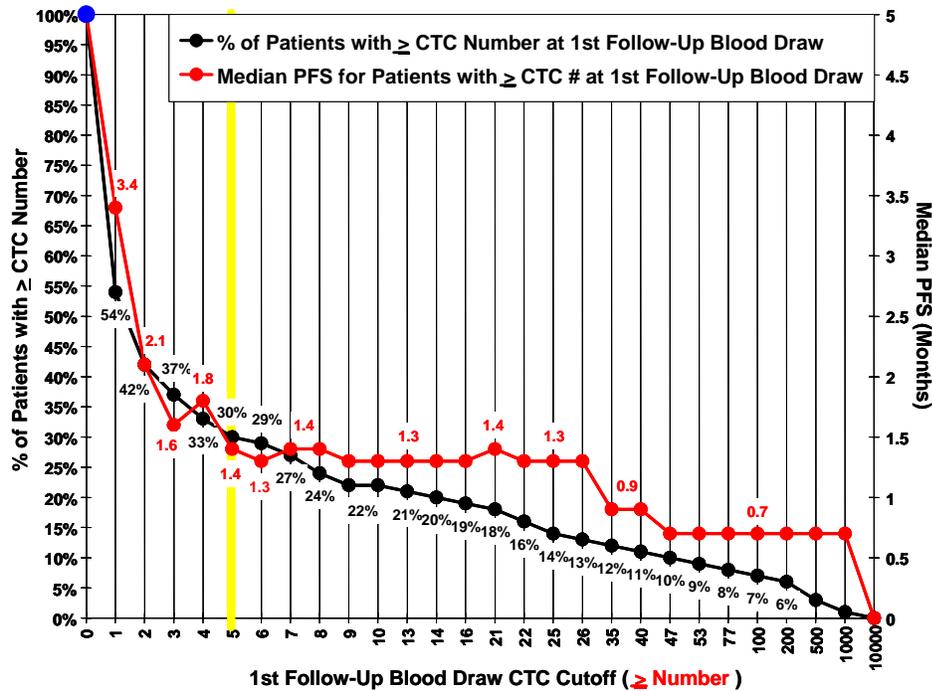


Figure 2 shows the median PFS for the positive patients (red line) and the percentage of positive patients (black line) for the Training Set at the cutoffs where either of these values changed. At the 5 CTC cutoff, 30% of patients are within the positive group and have a median PFS of 1.4 months.

Figure 2



The cutoff chosen also corresponded well with expected values determined with populations of healthy volunteers, persons with non-malignant breast disease, and persons with non-malignant other

disease. (See Table 3). 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 in the peripheral blood. 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 3**.

Table 3. 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-A2³

Based on the above data and analyses, the optimal CTC cutoff was determined to be at 5 CTC per 7.5 mL of blood. The FDA statistician, Harry Bushar, Ph. D., checked all of the statistical claims and agreed that the cutoff chosen by the sponsor was optimal.

2. Comparison studies:
 - a. *Method comparison with predicate device:*
Since the claimed predicate device was so different from the new device, no comparative studies were able to be performed.
 - b. *Matrix comparison:*
Since the only matrix claimed was blood, no matrix comparison studies were necessary nor performed.
3. Clinical studies:
 - a. *Clinical sensitivity:*
Not done. Kaplan-Meier analysis.
 - b. *Clinical specificity:*
Not done. Kaplan-Meier analysis
 - c. *Other clinical supportive data (when a and b are not applicable)*

Summary of Clinical Trial Results

Metastatic Breast Cancer Patients

A multi-center prospective, longitudinal clinical trial was conducted to determine whether trends in the number of CTCs correlates with disease progression. Only patients with measurable disease and who were starting therapy were enrolled (N=177). Patients were included on an intent to treat basis.

Clinical Trial Results

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, 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 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

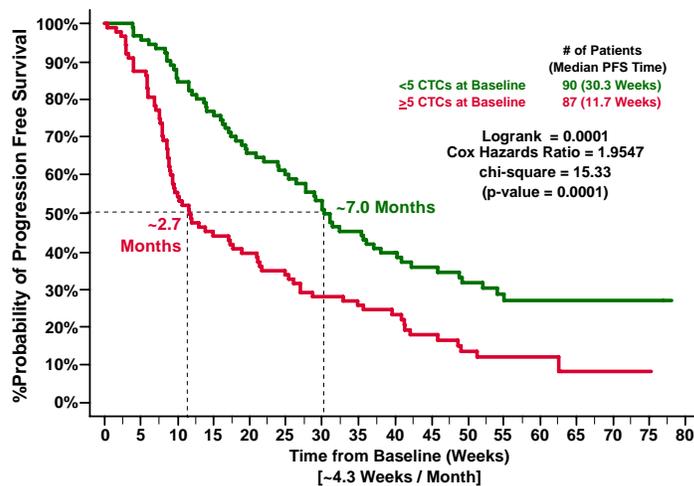
PFS Using Baseline CTC Results

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:

- Negative group (N=90), represented in **green**, was those patients with <5 CTC,
- Positive group (N=87), represented in **red**, was those patients with ≥ 5 CTC.

Median PFS was 30.3 weeks (~7.0 months) for the Negative group and 11.7 weeks (~2.7 months) for the Positive 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).



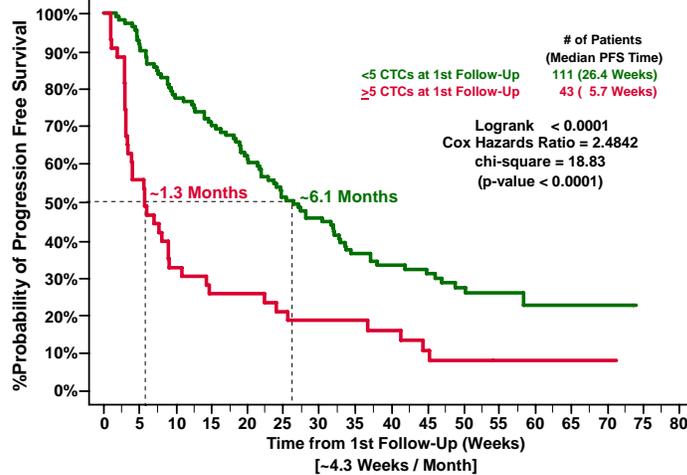
PFS Using 1st Follow-up CTC Results

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:

- Negative group (N=111), represented in **green**, was those patients with <5 CTC,
- Positive group (N=43), represented in **red**, was those patients with ≥ 5 CTC.

Median PFS was 26.4 weeks (~6.1 months) for the Negative group and 5.7 weeks (~1.3 months) for the Positive 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).



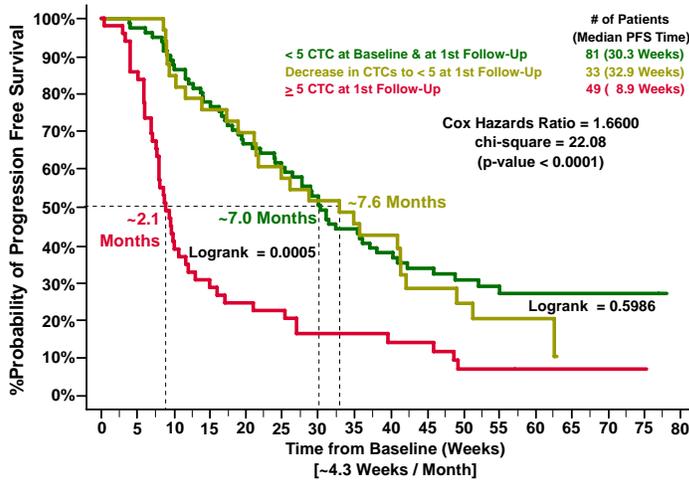
Predictive Value of CTC on PFS

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

- Negative group (N=81), represented in **green**, was those 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),
- Positive group (N=49), represented in **red**, was those 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 Negative 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 Positive 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 Negative and **olive green** groups compared to the PFS of the patients in the Positive group is highly significant (Log-rank $p \leq 0.0006$, 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)

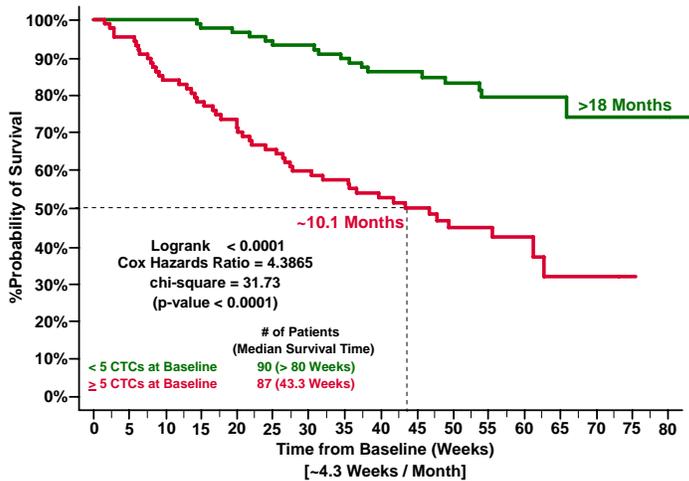


Overall Survival (OS) Analysis

OS Analysis Using Baseline CTC Results

At the time of these analyses, 66 (37%) of the 177 patients had died. Seventeen (19%) of 90 patients from Negative group (<5 CTC at baseline) compared to 49 (56%) of 87 from Positive Group (≥5 CTC at baseline) died. Median OS was greater than 80 weeks (>18 months) for the Negative group and 43.3 weeks (~10.1 months) for the Positive 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**.

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



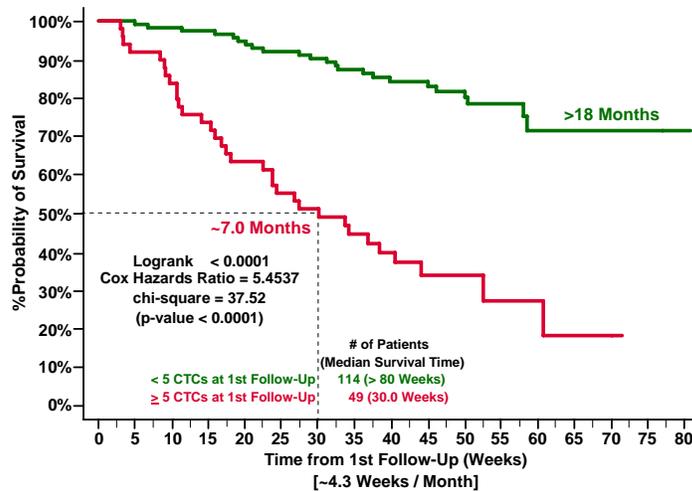
OS Using 1st Follow-up CTC Results

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

- Negative group (N=114), represented in **green**, was those patients with <5 CTC,
- Positive group (N=49), represented in **red**, was those patients with ≥5 CTC.

Fifty-six patients died of the 163 patients who were evaluable at the first follow-up. Twenty-three (20%) from the Negative group died with a median OS greater than 80 weeks (>18 months). Thirty-three (67%) from the Positive group died, with a median OS of 30.0 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 \geq 5 CTC at 1st Follow-Up (N=163).



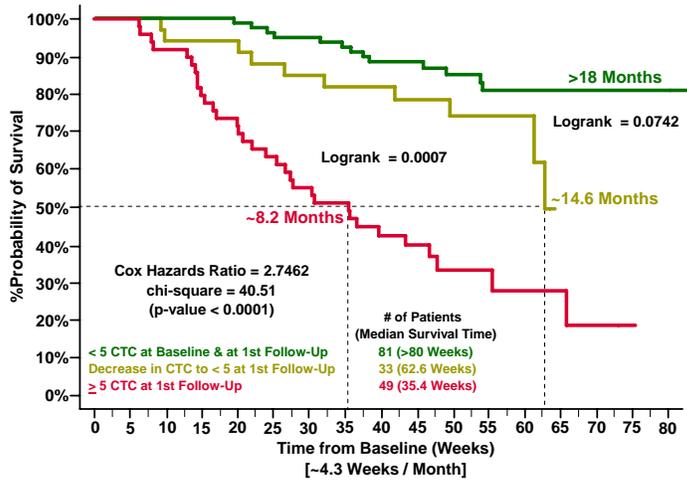
Predictive Value of CTC on OS

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

- Negative group (N=81), represented in **green**, was those 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),
- Positive group (N=49), represented in **red**, was those patients with \geq 5 CTC at 1st follow-up,

Figure 6 illustrates that a decrease to <5 CTC after the initiation of therapy significantly impacts OS. Elapsed OS time was calculated from the baseline blood draw. The three groups were plotted in **Figure 6**. The Negative group (**green** line) had a median OS of >80 weeks (18 months) and the patients represented by the **olive green** line (N=33) had a median OS of 62.6 weeks (~14.6 months). The Positive group (**red** line) had a median OS of 35.4 weeks (~8.2 months). This difference in the OS of the patients in the Negative and **olive green** groups compared to the OS of the patients in the Positive group is highly significant (Log-rank $p \leq 0.0007$, Cox Hazards Ratio=2.7462, chi-square=40.51, $p < 0.0001$). These data suggests that baseline and follow-up samples support the concept that repeated evaluation of CTC levels may provide ongoing information on the patients 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)



4. Clinical cut-off:
Please see 1f. above.
5. Expected values/Reference range:
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 in the peripheral blood. 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 3**.

Table 3. 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
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* NCCLS Guideline C28-A2³

M. Instrument Name:

CellSpotter™ Analyzer

N. System Descriptions:

1. Modes of Operation:

semi-automated

2. Software:

Operating System –

Windows NT 4.0

User Interface –

Primarily written in Visual Basic; Graphic User Interface (GUI)

Communication –

No communication in or out of the system;
 No LIMs or LIS System interface (no standards exist for this device)

Data Management –

DB2 database from IBM is used for data management

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types: Yes X

3. Sample Identification:
 Sample information is manually entered by user from printout from CellPrep
4. Specimen Sampling and Handling:
 Sample prepared on the CellPrep™ Analyzer and manually placed into instrument
5. Assay Types:
 Cytochemistry image analysis
6. Reaction Types:
 Fluorescence microscopy
7. Calibration:
 None
8. Quality Control:
 The *CellSearch*™ Control Cell Kit is provided by the manufacturer to control the system.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The “L. Performance Characteristics” Section Of The SE Determination Decision Summary.

Recovery

Blood from a single healthy donor was pooled and five 7.5 mL samples were spiked with cells of a breast cancer cell line (SKBr-3). Serial dilutions were performed resulting in twenty-five samples with expected cell counts ranging from 4 to 1142 cells per 7.5 mL. These samples were processed on CellPrep™ instruments, analyzed over five consecutive days, and the results of the observed cell counts were plotted against the results of the expected cell counts. The mean recovery of cells ranged from 85 to 123%. However, because of the low cell numbers and the inherent variation in spiking low numbers of cells, recovery at the low end of the range was imprecise. A difference of only 2 cells in the lowest dilution represents a 50% difference. 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 25 samples was $Y=0.85x + 5.64$, $R^2=0.9973$. Analysis of the regression data showed that the 95% confidence interval of the intercept of 5.64 was -4.421 to 15.69 , which overlaps zero and therefore is not statistically different from zero. This study suggests that the average recovery of spiked cells using the CellPrep™ instrument and the CellSearch™ Kit is approximately 85%.

P. Proposed Labeling:

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

Q. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.6020 with special controls. The special control guidance document "Immunomagnetic Circulating Cancer Cell Selection and Enumeration System" is available at WWW.fda.gov/cdrh/oivd/guidance/1531.pdf