510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k062013

B. Purpose for Submission:
To expand the intended use of the CellSearch Assay as an aid in the monitoring of patients with metastatic breast cancer in conjunction with other clinical methods

C. Measurand:
EpCam, Cytokeratins 8, 18 and/or 19, and CD45

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 (Epithelial)

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. The test is to be used as an aid in the monitoring of patients with metastatic breast cancer. Serial testing for CTC should be used in conjunction with other clinical methods for monitoring breast cancer. A CTC count of 5 or more per 7.5 mL of blood at any time during the course of the disease 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 (k040077) and the CellTracks® Analyzer II.

(k050145 and k060110) 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 consists of anti-EpCAM Ferrofluid (mouse monoclonal antibody (mAB) to EpCAM conjugated-magnetic nanoparticles in buffer with BSA and ProClin 300), staining reagent (phycoerythrin (PE)-conjugated mouse anti-cytokeratins mAB to and allophycocyanin (APC)-conjugated mouse anti-CD45 mAB in buffer with BSA and sodium azide), nucleic acid dye (4', 6-diamidino-2-phenylindole, dihydrochloride and ProClin 300),capture enhancing reagent, permeabilization reagent, cell fixative, dilution buffer, conical tubes and caps and cartridges and cartridge plugs.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   UroVysion Bladder cancer Recurrence Kit and Vitros Immunodiagnostic Products CA15-3 Assay
2. Predicate 510(k) number(s):
   k013785 (UroVysion) and k983690 (Vitros)
3. Comparison with predicate:

	CellSearch CTC Kit (k062013)	UroVysion Bladder Cancer Recurrence Kit (k013785)
Intended Use: Cancer Monitoring	The test is to be used as an aid in the monitoring of patients with metastatic breast cancer. Serial testing for CTC should be used in conjunction with other clinical methods for monitoring breast cancer. A CTC count of 5 or more per 7.5 mL of blood at any time during the course of the disease is predictive of shorter progression free survival and overall survival.	Results from the UroVysion Kit are intended for use as a noninvasive method for monitoring for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with bladder cancer.
Primary Cancer Site	Breast	Bladder
Measurand	CTC positive for EpCam, Cytokeratins 8, 18 and/or 19	Aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus
Assay Type	Cellular	Cellular
Technology	Immunomagnetic capture, enrichment and detection	Fluorescent in situ hybridization (FISH)
Fluorescent reagents	Anti-CK-PE, DAPI, and	Chromosome Enumeration Probe

	CellSearch CTC Kit (k062013)	UroVysion Bladder Cancer Recurrence Kit (k013785)
	anti-CD45-APC	(CEP®) 3 SpectrumRed^{TM}, CEP 7 SpectrumGreen^{TM}, CEP 17 SpectrumAqua^{TM}, and Locus Specific Identifier (LSI®) 9p21 SpectrumGold^{TM}.
Recognition of positive cells	Visual recognition through fluorescent signal	Visual recognition through fluorescent signal
Means of measurement	Fluorescent cell counting compared to cutoff value	Fluorescent cell counting compared to cutoff values
Cutoff for Progression/Survival	Crossing of one threshold value at any time point	Crossing of one of two threshold values at one time point

	CellSearch CTC Kit (k062013)	Vitros Immunodiagnostic Products CA 15-3 Assay (k983690)
Intended Use: Cancer Monitoring	The test is to be used as an aid in the monitoring of patients with <u>metastatic</u> breast cancer. Serial testing for CTC should be used in conjunction with other clinical methods for monitoring breast cancer. A CTC count of 5 or more per 7.5 mL of blood at any time during the course of the disease is predictive of shorter progression free survival and overall survival.	Serial test results obtained with the VITROS® CA 15-3 assay, in patients who are <u>clinically free of disease</u>, should be used in conjunction with all relevant information derived from diagnostic test, physical examination and full medical history in accordance with appropriate patient management procedures used for early detection of recurrence. The test is also intended for use as an aid in the management of breast cancer patients with <u>metastatic disease</u> by monitoring progression or response to treatment.
Primary Cancer Site	Breast	Breast
Sample Type	Whole blood	Serum, plasma (heparin or EDTA)
Technology	Immunomagnetic capture, enrichment and detection	Solid-phase immunoassay
Cutoff for Progression/Survival	Crossing of one threshold value at any time point	Significant difference between test results from two different time points

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)

"Guidance Document for the Submission of Tumor Associated Antigens Premarket Notifications, [510(k)], to FDA to Guide Manufacturers

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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance was presented originally in K031588:
   a. *Precision/Reproducibility:*
      i. **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%

ii.   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%

b. *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.

Linearity/assay reportable range:
Another way to examine the previous data is to analyze it as a dilution series to evaluate test linearity. The confounding variable of percent recovery was removed 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:*
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 (k031588).

2. Comparison studies:
 a. *Method comparison with predicate device:*
Direct comparison to the UroVysion Bladder cancer Recurrence Kit is not feasible since it is different measurands in a different intended population. As for the Vitros CA15-3 assay, the measurand is a serum tumor marker and not for circulating tumor cells.
 b. *Matrix comparison:*
Since there is only one matrix for this test, i.e. whole blood, 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):*

USE OF CTC TO MONITOR CLINICAL STATUS

Relationship between survival, CTCs, and disease assessment by imaging
Radiological imaging is one of the primary means of determining disease status and response to therapy in metastatic breast cancer patients. To establish the relationship of CTCs, measured at two different time points, to clinical status as determined by imaging, CTCs and imaging results were compared 1) to the true clinical endpoint overall survival and 2) to each other.

CTC
Previous data has shown that patients with ≥5 CTCs / 7.5mL of blood at any succeeding follow-up visit after the initiation of therapy had a higher likelihood of progressive disease and decreased overall survival compared to patients with <5 CTCs / 7.5mL of blood.

Imaging
All imaging sites were in compliance with Digital Imaging and Communications in Medicine (DICOM) standards. Using standardized digital images, two expert radiologists (readers), working individually and blinded to clinical information, classified each follow-up disease assessment (total of 231 imaging studies) from 138 patients with measurable disease as indeterminate (I), stable disease (S), partial response (PR), or progressive disease (PD) according to World Health Organization (WHO) bi-dimensional criteria. Measurable disease was defined as the presence of at least one lesion ≥2cm in its longest dimension. Readers identified up to eight lesions per patient per time point by describing the longest dimension of the lesion and the longest perpendicular dimension. These two dimensions were multiplied and the "cross product" was reported. Summed measurements for the cross-products were calculated, and percent change from the previous time point was determined. Although all patients had measurable disease, non-measurable lesions (still detectable by radiology) were included in the determination of patient status as described in the WHO guidelines. Progressive disease was defined as a >25% increase in the sum of all lesions or appearance of a new measurable or non-measurable lesion. Partial response was defined as a decrease in the sum of all lesions of ≥50% and no new lesions.

- Radiology interpretations from the two expert radiologists were classified as followed:
    - S and PR were considered to both reflect non-progressive disease (NPD)
    - PD was considered to reflect progressive disease
    - In situations where one of the radiologists rendered a classification of Indeterminate (I) but the other radiologist rendered a classification of S, PR or PD, the classification of the latter radiologist was used for comparison to CTCs (n=11)
    - When both radiologists rendered a classification of Indeterminate (I), then the data was not used in the comparison to CTCs (n=3)
    - A third independent radiologist adjudicated disagreements between the two primary readers regarding PD and NPD (n=27)

- In situations where the third independent radiologist rendered a classification of Indeterminate (I), the data was not used in the comparison to CTCs (n=2)
- In serial imaging studies, radiology results that were less than one month from a previous tabulated observation were not used (n=1).
- The CTC results obtained within ± one month of the imaging study were classified as <5 CTC and ≥5 CTC. If more than one CTC value was obtained within ± one month of the imaging study, the CTC result obtained closest to the date of the imaging study was used.

Concordances between CTC and Radiological Monitoring

Imaging studies are a major component of the current standard of care for determining disease progression and response to treatment in the metastatic breast cancer setting. To support the effectiveness of CTCs in making these clinical assessments, two-by-two tabulations of concordant and discordant observations between CTCs and radiological imaging were constructed using the previously described criteria.

Using only the 1st follow-up imaging study, the radiological response at this visit was compared with the CTC results obtained within ± one month of this imaging study. A total of 134 of the 138 patients (97%) had CTC results that met this criterion. The result of this "patient-wise" comparison between CTCs and imaging is shown in **Table 4**.

Table 4. Patient-Wise Comparison of CTC and Imaging

Response at 1st Follow-Up Imaging Study	CTCs within ± 1 Month of Imaging		Total
	<5 CTCs/7.5mL	≥5 CTCs/7.5mL	
Non-Progressive Disease	85	9	**94**
Progressive Disease	20	20	**40**
Total	**105**	**29**	**134**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement	50%	34%	66%
Negative % Agreement	90%	83%	96%
Positive Predictive Value	69%	49%	85%
Negative Predictive Value	81%	72%	88%
Overall Agreement	78%	70%	85%
Odds Ratio	9.4	3.4	26.8

Using all of the follow-up imaging studies performed after the initiation of therapy on the 138 patients that rendered useable radiological response results (n=225), these results were then compared to CTC results obtained within ± one month of the imaging study. A total of 219 of the 225 (97%) imaging studies had CTC results meeting this criterion. The result of this "observation-wise" comparison between CTCs and imaging is shown in **Table 5**.

Table 5. Observation-Wise Comparison of CTC and Imaging

Response at All Follow-Up Imaging Studies	CTCs within ± 1 Month of Imaging		Total
	<5 CTCs/7.5mL	≥5 CTCs/7.5mL	
Non-Progressive Disease	151	16	**167**
Progressive Disease	30	22	**52**
Total	**181**	**38**	**219**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement	42%	29%	57%
Negative % Agreement	90%	85%	94%
Positive Predictive Value	58%	41%	74%
Negative Predictive Value	83%	77%	89%
Overall Agreement	79%	73%	84%
Odds Ratio	6.9	3.0	15.8

In serial observations, only a minority of the transitions for imaging results between non-progressive disease and progressive disease coincided with a matching transition of CTC counts between <5 and ≥5 CTCs/7.5 mL.

Because the prognostic value of the CTC results at an earlier time-point were equivalent to that of the CTC results at the time of imaging, a patient-wise comparison using results from only the 1st follow-up imaging study, performed approximately 9 weeks after the initiation of therapy, and the CTC results obtained at the 1st follow-up, performed approximately 4 weeks after initiation of therapy, was constructed. All 138 patients had CTC results meeting this criterion. The result of this "patient-wise" comparison between CTCs at an earlier time point and imaging is shown in **Table 6**.

Table 6. Patient-Wise Comparison of CTC and Imaging

Response at 1st Follow-Up Imaging Study	CTCs at 1st Follow-Up		Total
	<5 CTCs/7.5mL	≥5 CTCs/7.5mL	
Non-Progressive Disease	84	12	**96**
Progressive Disease	20	22	**42**
Total	**104**	**34**	**138**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement	52%	36%	68%
Negative % Agreement	88%	79%	93%
Positive Predictive Value	65%	46%	80%
Negative Predictive Value	81%	72%	88%
Overall Agreement	77%	69%	84%
Odds Ratio	7.7	3.0	19.9

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 (k031588).
5. Expected values/Reference range:
   The EXPECTED VALUES were determined with a previous version of this test , as presented in k031588. The present version of the test was demonstrated to be substantially equivalent to that in k031588 in k050245.

   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 7**.

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.