

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

K050145

B. Purpose for Submission:

Redesign of the instrument configuration to place the fluorescent microscope, camera, sample handling mechanism (stage), mercury arc lamp and power supply within a single housing with replacement of obsolete components (e.g., camera) by the same manufacturer of both instruments, Immunicon Corporation.

C. Manufacturer and Instrument Name:

Immunicon Corporation's CellTracks Analyzer II

D. Type of Test or Tests Performed:

A semi-automated qualitative immunomagnetic-capture immunofluorescent detection image analysis fluorescence microscope used to aid the CLIA compliant CellTracks trained testing personnel in the enumeration of circulating tumor cells (CTC) (cells appearing like tumor cells with epithelial cell markers and no lymphocyte marker on their surfaces).

E. System Descriptions:

1. Device Description:

The *CellTracks®* Analyzer II is a semi-automated fluorescence microscope. The product consists of the *CellTracks®* Analyzer II, a dedicated computer loaded with *CellTracks®* software, monitor, keyboard and mouse.

2. Principles of Operation:

The *CellTracks®* Analyzer II is used in conjunction with the *CellTracks®* AutoPrep System and reagent kits that contain a ferro-fluid-based capture reagent and immunofluorescent reagents for detection and characterization of the captured cells. The ferrofluid reagent consists of nano-particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the cells of interest. For example, the CellSearch™ Circulating Tumor Cell Kit (available from Veridex, LLC, a Johnson and Johnson company) contains anti-EpCam ferrofluid that targets the Epithelial Cell Adhesion Molecule for capturing circulating tumor cells (CTCs), whereas the *CellTracks®* Endothelial Cell Kit contains anti-CD146 ferrofluid to capture CD146+ cells. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of the target cells.

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. Cell images from each filter are compiled and presented in a gallery format for final cell classification by

the user.

3. Modes of Operation:
Semi-automated one-at-a-time MagNest® analysis.
4. Specimen Identification:
Identification information entered individually for each patient sample.
5. Specimen Sampling and Handling:
The MagNest® is not mixed or pierced, but put into place manually and then the surface is automatically scanned. The CLIA compliant CellTracks trained testing personnel then manually interprets the results.
6. Calibration:
Insert the *CellTracks*® System Verification Cartridge into its holder, enter the cartridge ID number, place into the *CellTracks*® Analyzer II, close the door and click the OK button. A progress bar is displayed while system verification is in progress. The *CellTracks*® System Verification Cartridge contains a strip of material impregnated with dyes. System verification checks the optical performance and chamber “skew” which ensures proper scanning. There is no recognized reference material or method.
7. Quality Control:
For the circulating tumor cell assay the CellSearch® Circulating Tumor Cell Control Kit should be run once per day of patient testing as a patient sample following each user’s quality assurance program.
8. Software:
FDA has reviewed applicant’s Hazard Analysis and Software Development processes for this line of product types:
Yes X or No
The software submission was reviewed and found to be acceptable for a moderate hazard level.

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:
Immunology (82)

G. Intended Use:

1. Indication(s) for Use:
The Immunicon CellTracks Analyzer II is a semi-automated fluorescence microscope used to enumerate fluorescently labeled cells that are immunomagnetically selected and aligned. The product is for *in vitro* diagnostic use when used in tandem with specimen preparation equipment and reagents that are legally marketed for *in vitro* diagnostic use with this device.
2. Special Conditions for Use Statement(s):
For prescription use only

H. Substantial Equivalence Information:

1. Predicate Device Name(s) and 510(k) numbers:
Veridex LLC CellSearch™ Epithelial Cell Kit/Cell Spotter Analyzer. K031588
2. Comparison with Predicate Device:

| Similarities | | |
|----------------------------------|--|------------------------------------|
| Item | Device | Predicate |
| Name | CellTracks Analyzer II | CellSpotter Analyzer |
| Sample | Processed via MagNest® fixture | Processed via MagNest® fixture |
| Available channels for analysis | 4 | 4 |
| Mercury Arc Lamps Manufacturer | USHIO | Same |
| Lamp Alignment | Manual | Same |
| 10x Objective | Nikon | Same |
| Lamp Stabilization Time | 15 minutes | Same |
| Verification Stations | DAPI | Same |
| Computer Operating System | XP | Same |
| Browser | Netscape | Netscape |
| Scan process | 1 cube/position | Same |
| | | |
| Differences | | |
| Item | Device | Predicate |
| Name | CellTracks Analyzer II | CellSpotter Analyzer |
| Applicant | Immunicon Corporation Huntingdon Valley, PA | Veridex, LLC Warren, NJ |
| Camera Manufacturer ¹ | DVC | Hamamatsu |
| Type | Monochrome | Monochrome |
| Total Number of Pixels | 1434 (H) x 1050 (V) 1.5M pixels | 1360 (H) x 1034 (V) 1.4M pixels |
| Effective Pixels | 1393 (H) x 1040 (V) 1.45M pixels | 1300 (H) x 1030 (V) 1.3M pixels |
| Chip Size | 10.2mm (H) x 8.3mm (V) | 10.0mm (H) x 8.7mm (V) |
| Unit Cell Size | 6.45µm (H) x 6.45µm (V) | 6.7µm (H) x 6.7µm (V) |
| Cartridge Frames Scanned | 175 | 140 or 175 |
| Bulb Replacement | Operator | Service |
| ND Filters ² | Automatic | Manual |
| Edge Focus ³ | Automatic | Manual |
| Computer | IBM 64 Bit | IBM 32 Bit |
| Monitor | 23" Wide Screen/ | 17" - 19" Square/ |

| Similarities | | |
|-------------------|--|--|
| Item | Device | Predicate |
| | Resolution 800 x 600 | Resolution 800 x 600 |
| Microscope Lenses | All the same with the following exception ⁴ | All the same with the following exception ⁴ |
| APC Filter | All the same with the following exception ⁵ | All the same with the following exception ⁵ |

¹Changes in camera precipitated due to Hamamatsu camera no longer being available. The Sony ICX285 AL is the successor to the Sony ICX085 used in the CellSpotter camera. The increase in total number of pixels will result in higher resolution. The increase in the quantum efficiency allows the system to measure more signal therefore requiring less power and eliminating the need for a rear reflector.

² The change to automatic ND Filter and Edge Focus in CellTracks® Analyzer II eliminates the Joystick control box on the CellSpotter® Analyzer. The CellTracks® Analyzer II automates the ND filter process by actuating the filter at the proper times in the instrument setup, thus eliminating the potential for operator error of leaving the filter in place during a scan of a specimen.

³ Edge Focus is semi-automated by moving the stage to the proper location and allowing the operator to determine the edge of a long and short axis of the cartridge. The opposite long and short access does not have to be found because of algorithms in the software. On CellTracks® Analyzer II, the operator moves a line to the edge of the cartridge using the arrow keys of the computer keyboard.

⁴ CellTracks® Analyzer II does not require all the features the Nikon attachment offers because the CellTracks® Analyzer II requires a fixed focal plane dimension on the cartridge provided by the negative lens. The Nikon attachment allows for a variable focal plane. The CellTracks® Analyzer II design has less lens elements reducing the light loss of the assembly by reducing the number of optical surfaces.

⁵ For the APC filter, CellTracks® Analyzer II will have narrower bandpass wavelengths to increase APC detection and reduce PE spillover. The differences are:

| APC Bandpass | CellTracks® Analyzer II (submission device) | CellSpotter Analyzer (Predicate) |
|---|--|-------------------------------------|
| Lower range Wavelength/tolerance – mean wavelength – Upper range wavelength/tolerance (all units in nm) | 620/30 – 647 – 675/50 ~ 80% | 620/60 – 660 – 700/75 ~ 77 % |

I. Special Control/Guidance Document Referenced (if applicable):

The CellTracks was developed in conformance to the following standards.

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

FDA Guidance: General Principles of Software Validation

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.

In addition, the CellTracks Analyzer II will conform to the following two standards prior to marketing.

IEC 61010-1 IEC: Safety requirements for Electrical Equipment for Measurement, Control and Laboratory Use Part 1

BS EN61326 Electrical Equipment for Measurement, Control and Laboratory Use - EMC requirements.

J. Performance Characteristics:

All of the following performance characteristics were generated using the Veridex CellSearch™ Circulating Tumor Cell Kit (Epithelial). (K050245)

1. Analytical Performance:

a. *Accuracy*

(1) Comparison Study of New Version of Device to Predicate using Clinical Samples

A comparison study was performed using whole blood samples collected in CellSave® preservative tubes from cancer patients to determine circulating cancer cell counts. The samples were obtained from thirteen geographically dispersed sites and analysis was performed by Medical Technologists. The study compared the CellTracks Analyzer II to the CellSpotter predicate device. The Pearson's correlation coefficient for 83 specimens with an average of ≥ 1.5 circulating tumor cells was 0.9996 with a linear regression slope of 1.136 and an r^2 of 0.9992.

(2) Comparison Study of New Version of Device to Predicate using Tissue Comparison Study of New Version of Device to Predicate using Tissue Cultured Cell-Line Samples

To directly demonstrate comparable performance between the new system 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.

(3) EP9-A Comparison Study with SkBr-3 Tissue Culture Cells

A comparison was made of the performance of the CellTracks Analyzer II system for the detection of tumor cells from whole blood versus the CellSpotter® Analyzer (K031588). This method comparison was conducted in accordance with NCCLS EP9-A, Method Comparison and Bias Estimation Using Patient Samples, using whole blood from normal donors spiked with tissue culture cells (SKBr-3) at tumor cell counts that cover the clinical range. The range of tumor cells observed in this experiment was from 0 to 1960. Linear regression analysis showed the slope of the CellTracks analyzer II tumor cell count versus the CellSpotter analyzer cell count regression line = 1.03 with an intercept of -1.25 and an $r^2 = 0.9998$.

b. 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-A². 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.

Summary of Precision Analyses

| | <i>Low</i> | High |
|--|------------|-------------|
| N | 99 | 99 |
| Mean cell count | 48 | 969 |
| Total Precision Standard Deviation (S _T) % CV | 18% | 5% |

c. Linearity/Recovery:

(1) *Recovery Study:*

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 the following table.

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.

(2) Linearity / Reportable Range

Another way to examine the recovery data (see above) 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.

d. *Carryover:*

Specimens with more than 5,000 CTC per 7.5 mL of blood were less than 0.03% of those seen in our clinical studies. Sample carryover is of concern when such a high CTC specimen is immediately followed in the CellTracks® AutoPrep System by a specimen yielding a CTC result in the range 5 to 15 CTC per 7.5 mL of blood. In this case, we recommend obtaining a new blood sample from the low CTC patient and performing a confirmatory CTC analysis. To identify following samples, refer to the CellTracks® AutoPrep User's Guide section on View Data and obtain the detailed batch data, including sample and patient identification for each tube in the batch.

e. *Interfering Substances:*

Assaying with the CellSearch™ Epithelial Cell Kit, 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.

2. Other Supportive Instrument Performance Data Not Covered Above:

None

K. Proposed Labeling:

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

L. Conclusion:

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