

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

K030596

B. Analyte:

Blood Collection Tube

C. Type of Test:

Collection and preservation of cells

D. Applicant:

Immunicon Corporation

E. Proprietary and Established Names:

Immunicon CellSave™ Preservative Tube

F. Regulatory Information:

1. Regulation section:
862.1675; Tubes, vials, systems, serum separators, blood collection
2. Classification:
Class II
3. Product Code:
JKA
4. Panel:
75

G. Intended Use:

1. Indication(s) for use:
CellSave tubes are evacuated blood collection tubes that are designed to be used with standard phlebotomy supplies for venous blood collections for professional use. The tube contains 300µl of a solution that contains Na₂EDTA and a cell preservative. The preservative preserves morphology and cell surface antigen expression of the epithelial cells and leukocytes. This tube may be used for monitoring of circulating epithelial cells (tumor cells) which may aid in the management of cancer patients. This tube may be used for monitoring CD3+/CD4+ T lymphocyte subsets which may aid in the management of patients with HIV/AIDS.
2. Special condition for use statement(s):
3. Special instrument Requirements:

H. Device Description:

CellSave™ Tubes are evacuated blood collection tubes that contain EDTA anticoagulant and a cell preservative. The vacuum is designed to draw approximately 10 ml of blood. The interior of the tube is sterile.

I. Substantial Equivalence Information:

1. Predicate device name(s):

BD Vacutainer® Blood Collection tube with EDTA

2. Predicate K number(s):
Pre-amendment
3. Comparison with predicate:

Similarities		
Item	CellSave™ Tube	Vacutainer® Tube
Sample	whole blood	whole blood
Tube type	glass	glass
Sterile	yes	yes
Differences		
Item	CellSave™ Tube	Vacutainer™ Tube
Function	White blood cell and epithelial cell preservation	Blood specimen preservation
Cap color	Mottled lavender and yellow	lavender
Contents	Disodium EDTA and preservative	Dipotassium EDTA

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

The CellSave™ Sample Tube was tested and met the applicable requirements of ISO 6710 Single Use containers for venous blood specimen collection and NCCLS Standard H1-A4 Evacuated Tubes and Additives for Blood Specimen Collection- Fourth Edition; Approved Standard.

K. Test Principle:

CellSave™ Tubes are evacuated blood collection tubes that are designed to be used with standard phlebotomy supplies for venous blood collection. The tube contains 300 uL of a solution that contains Na₂EDTA and a cell preservative. The EDTA absorbs calcium ions, which prevents the blood from clotting. The preservative preserves the morphology and cell surface antigen expression of the epithelial cells and leukocytes. Each tube is evacuated to withdraw 10.0 ml of venous whole blood when following standard phlebotomy procedures. Studies indicated that within a couple of hours epithelial cell counts decreased significantly when preserved in EDTA alone. It was also observed that separation of leukocyte sub-populations identified by cell surface antigens as measured by flow cytometry decreased over time. The use of the preservative contained within the CellSave™ tube preserved circulating epithelial cells for 72 hours and maintained the separation of the leukocyte sub-populations for 24 hours.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*
NA
 - b. *Linearity/assay reportable range:*
Recovery was evaluated by spiking samples with low tumor cell numbers (0, 50, 100, and 200 cells/7.5 ml) and high tumor cell numbers (0, 100, 1000, and 10,000 cells/7.5 ml). Blood from 5 normal donors was collected into CellSave™ tubes and spiked with

SKBR-3 cells (a breast cancer cell line). Samples were processed and stained with a nucleic acid dye, anti-CD45-APC and anti-CK-PE using the CellPrep™ Semi-Automated Processing System and analyzed using a FACSCalibur flow cytometer with beads to enable calculation of absolute counts of cells. For the low spike experiment, the regression equation was $y=0.8x+4.7$ and the $R^2=0.98$. For the high spike experiment, the regression equation was $y=0.9x+6.2$ and the correlation was $R^2=0.99$.

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

NA

d. *Detection limit:*

NA

e. *Analytical specificity:*

Blood from 5 normal donors was collected into EDTA and CellSave™ tubes and spiked with approximately 800 SKBR-3 cells. CellSave™ tubes were spiked with potential interfering substances (hemolysis 5+, lipemia 1.94-2.04% emulsified fat, icteris 7.0 mg/dl) to determine the effect on recovery and enumeration of tumor cells. Duplicate samples were processed using CellPrep™ Semi-Automated Sample Processing System and analyzed using the FACSCalibur flow cytometer. Hemolysis, lipemic and icteric whole blood samples collected into the CellSave™ tube do not interfere with the recovery and enumeration of tumor cells

f. *Assay cut-off:*

NA

2. Comparison studies:

a. *Method comparison with predicate device:*

Blood from 11 donors was drawn in both EDTA and CellSave tubes for comparison at <2 hours, 24 hours in-house, and 24 hour shipping overnight. Samples were stained and analyzed on a flow cytometer. The geometric mean fluorescence intensity of CD45-PerCP staining of lymphocytes and granulocytes and CD4-PE staining of lymphocytes and monocytes were determined. In the comparison study using EDTA tubes and CellSave tubes, the CellSave tubes provided continued improved separation between lymphocytes and granulocytes using CD45-PerCP reagent as compared with cells in EDTA. The geometric mean fluorescence intensity (MFI); the ratio between the two; and the CVs of these measurements are improved when using the CellSave tube, as summarized in the tables below.

CD45 Antigen Density Table

CD45 antigen density of Lymphocytes and granulocytes

Sample	EDTA				CellSave™			
	CD45 antigen density				CD45 antigen density			
	Lymphs MFI	Grans MFI	Ratio L/G	CV%	Lymphs MFI	Grans MFI	Ratio L/G	CV%
Fresh	869.1	138.4	6.7	27%	817.5	89.7	9.3	16%
Inhouse	790.5	222.3	3.8	22%	640.2	66.5	9.9	14%
Shipped	798.3	224.6	3.7	21%	632.9	65.0	9.9	12%

CD4 Antigen Density Table

CD4 antigen density of Lymphocytes and monocytes

Sample	EDTA CD4 antigen density				CellSave™ CD4 antigen density			
	Lymphs MFI	Monos MFI	Ratio L/M	CV%	Lymphs MFI	Monos MFI	Ratio L/M	CV%
Fresh	770.7	93.2	8.4	14%	645.3	51.2	12.6	12%
Inhouse	665.8	196.7	3.4	14%	469.8	34.2	13.8	11%
Shipped	717.3	217.1	3.4	25%	489.1	36.0	13.6	12%

b. *Matrix comparison:*

NA

3. Clinical studies:

a. *Clinical sensitivity:*

NA

b. *Clinical specificity:*

NA

c. *Other clinical supportive data (when a and b are not applicable):*

A Clinical study was performed at Immunicon using blood samples obtained from five geographically dispersed sites from 102 metastatic cancer patients providing 107 blood specimens for analysis. Seventy of these specimens had sufficient blood volume for testing at approximately 24 hours and again at approximately 96 hours. Initial circulating epithelial cell (tumor cell, or CTC) counts ranged from 0 to 640 CTC per sample. Twenty-one of these specimens had an average of greater than or equal to 3 CTC at the two testing time points. The linear correlation for CTC recovery over this time period comparing 24 hours to 96 hours was an R^2 equal to 1.00 with a regression equation of $y=1.1x-7.1$. A Wilcoxon signrank test indicated that the CTC counts obtained at 24 hours and those obtained at 96 hours were not significantly different ($p > 0.90$). These data demonstrate that the recovery of circulating epithelial cells from whole blood remains stable over a 72 hour time period using the *CellSave*™ blood collection tube.

A second clinical study was performed to compare CD3/CD4 immunophenotyping over a 72 hour time period using both EDTA and *CellSave*™ tubes. Whole blood samples were obtained from fifty healthy volunteers and twelve patients with confirmed HIV. Results of CD3/CD4 testing on days 1, 2, and 3 resulted in R^2 values during the three days of between 0.97 and 0.98 and slopes of 0.91 to 0.97.

Together, these studies demonstrate that the *CellSave*™ blood collection tube is effective in preserving T lymphocytes and epithelial cells for phenotyping and enumeration over a 72 hour

time period. The numbers of CD3 and CD4 positive lymphocytes and epithelial cells are unchanged over a 72-hour period. Preservation of T lymphocytes and their antigens are also effective using different instruments for enumeration of labeled cells, which demonstrates that the *CellSave*[™] tube is useful across multiple instruments.

These studies justify the use of the CellSave tube for drawing, shipping and storing venous blood up to 72 hours for the counting and immunophenotyping of epithelial cells and leukocytes.

4. Clinical cut-off:
NA
5. Expected values/Reference range:
NA

M. Conclusion:

Based upon review of the information provided in this 510(k), I recommend that this device is substantially equivalent to devices regulated by 21 CFR 809.10; and regulations 21 CFR 862.1675, tubes, vials, systems, serum separators, blood collection; 75 JKA; Class II.