510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K162723

B. Purpose for Submission:

New Device

C. Measurand:

Leukocyte subsets

D. Type of Test:

Qualitative and Quantitative Flow Cytometry

E. Applicant:

Caltag Medsystems Ltd.

F. Proprietary and Established Names:

TransFix®/EDTA Vacuum Blood Collection Tubes

G. Regulatory Information:

1. Regulation section:

   21 CFR § 862.1675– blood specimen collection device

2. Classification:

   Class II

3. Product code:

   GIM

4. Panel:

   Clinical Chemistry and Toxicology (75)

H. Intended Use:

1. Intended use:

   Transfix/EDTA Vaccum Blood Collection Tubes (TVTs) are intended for collection and storage of human whole blood specimens for immunophentoyping of white blood cells by flow cytometry. Recovery of lymphocyte subset marker of the HIV panel can be accomplished over a 14-day period following collection.

   TVTs are in-vitro diagnostic medical devices.

2. Indication for use:

   Same as Intended Use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

TransFix/EDTA Vacuum Blood Collection Tubes (TVTs) are intended for collection and storage of human whole blood specimens for immunophenotyping of white blood cells (WBC, leukocytes) by flow cytometry up to 14-days following collection; the TVTs preserve the qualitative and quantitative leukocyte subset characteristics. Flow cytometry is used to identify subsets of leukocytes that can be distinguished on the basis of cell surface antigens using fluorescent antibodies.

The TransFix®/EDTA Vacuum Blood Collection Tubes (TVTs) consist of purple-capped polyethylene terephthalate blood collection tubes containing the paraformaldehyde based preservative, TransFix, and K3EDTA at the correct volume to simultaneously stabilize and anti-coagulate human whole blood at the time of collection. TVTs hold three mLs (final draw volume) with a tube dimension of 13 x 75mm. TVTs are sterilized by gamma radiation. Further, the vacuum contained within the TVT ensures that the TransFix/EDTA reagent is administered at the correct ratio of one part TransFix/EDTA to five parts whole blood.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Cyto-Chex® BCT

2. Predicate 510(k) number(s):

   K080552

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	TransFix/EDTA Vacuum Blood Collection Tubes (TVTs) are intended for collection and storage of human whole blood specimens for immunophenotyping of white blood cells by flow cytometry. Recovery of lymphocyte subset markers of the HIV panel can be accomplished over a 14-day period following collection.	Cyto-Chex® BCT is intended for the collection and storage of blood specimens for immunophenotyping of WBC by flow-cytometry. Recovery of lymphocyte subset cell markers of the HIV panel can be accomplished over a 14-day period following collection.
Preservation of HIV markers	14 days	Same
Tube size	13x75mm	Same

Differences		
Item	Device	Predicate
Tube type	Polyethylene terephthalate	Glass
Sample volume	2.5mL	5 mL
Liquid reagent	0.5mL	75.8µl
Contents	Liquid reagent: K3EDTA and TransFix preservative	Liquid reagent: K3EDTA and preservative

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

Not applicable

L. Test Principle:

Lymphocyte subsets are distinguished by the cell surface antigens that are detected through the use of fluorochrome-labeled antibodies and flow cytometry instruments. Qualitative and quantitative changes in lymphocyte subsets are used to identify and monitor immunodeficiency and hematological diseases. TransFix®/EDTA Vacuum Blood Collection Tubes are designed to preserve peripheral blood sample qualitative and quantitative lymphocyte subset characteristics.

M. Performance Characteristics (if/when applicable):

1. Analytical performance using the Multitest™ IMK kits (K974360 and K980858):

   All studies met the manufacturers predetermined acceptance criteria.

   a. Precision/Reproducibility:

   Lot to Lot Reproducibility

   Peripheral whole blood was collected from five healthy subjects in two TVTs from three lots at one site. The peripheral whole blood was tested at the same site on Days 0 (<6 hours from venipuncture), 12, and 15. Blood samples were stored at 2–8°C immediately after testing on Day 0 and in between test Days 12 and 15. At each time point, testing was performed with triplicate determinations on each of the duplicate TVTs from each of the three lots of investigational product. For each sample, all six CD markers were tested (CD3, CD8, CD4, CD45, CD16/CD56, CD19) using a BD FACs Calibur (K973483).

CD3+		**Within-tube**		**Between-Day**		**Between-Lot**		**Between-Replicate**		**Total**	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	1470.3	46	3.1	49.2	3.3	52.2	3.5	14.6	1	89.9	6.1
2	1183.6	28.5	2.4	46	3.9	41.4	3.5	16.4	1.4	72.8	6.2
3	404.7	2.7	0.7	7.1	1.8	10.4	2.6	6.1	1.5	19.4	4.8
4	1334.9	32	2.4	5.9	0.4	35.8	2.7	7.3	0.5	62.4	4.7
5	1458.9	22	1.5	25.7	1.8	62.9	4.3	16.4	1.1	82.5	5.7

CD8+		Within-tube		Between-Day		Between-Lot		Between-Replicate		Total	
Sample	Mean	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	517.7	12.5	2.4	44.7	8.6	29.2	5.6	1.4	0.3	56	10.8
2	451.2	8.1	1.8	25.4	5.6	19.6	4.4	5.2	1.1	34.1	7.6
3	167.7	3.6	2.2	8.6	5.2	11.3	6.7	3.1	1.8	16.6	9.9
4	758.4	7.4	1	11.2	1.5	20.2	2.7	4.5	0.6	46.8	6.2
5	572.1	8.8	1.5	3.4	0.6	27.1	4.7	11	1.9	40.2	7

CD4+		Within-tube		Between-Day		Between-Lot		Between-Replicate		Total	
Sample	Mean	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	767.5	21.6	2.8	29.9	3.9	29.5	3.8	12.5	1.6	59	7.7
2	685	11.6	1.7	25.4	3.7	7.9	1.2	9.3	1.4	44.5	6.5
3	197.9	0.8	0.4	7.2	3.7	0.9	0.5	2.3	1.2	16	8.1
4	496.3	8	1.6	11.9	2.4	11.4	2.3	4.9	1	35	7.1
5	828.2	7.1	0.9	6.9	0.8	24.8	3	23.7	2.9	52.4	6.3

CD45+		Within-tube		Between-Day		Between-Lot		Between-Replicate		Total	
Sample	Mean	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	2306.7	72	3.1	70.3	3	67.7	2.9	18.3	0.8	127.1	5.5
2	1730.2	29.7	1.7	49.3	2.8	63.5	3.7	26.1	1.5	97.1	5.6
3	720.5	9.6	1.3	9.9	1.4	27.7	3.8	11.3	1.6	37.5	5.2
4	1683	34.3	2	6.7	0.4	34.2	2	10.4	0.6	75.8	4.5
5	1952	34.4	1.8	19.3	1	88.5	4.5	20.2	1	107.8	5.5

CD16/56+		Within-tube		Between-Day		Between-Lot		Between-Replicate		Total	
Sample	Mean	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	377.1	11.6	3.1	12.3	3.3	0.1	0	2.3	0.6	24.9	6.6
2	366.7	4.3	1.2	15.9	4.3	21	5.7	8	2.2	31.1	8.5
3	209.5	5.2	2.5	6.4	3.1	15.4	7.4	3.4	1.6	17.2	8.2
4	217.7	4.2	1.9	6.4	3	5.1	2.3	3.9	1.8	15.5	7.1
5	296.9	9.6	3.2	11.5	3.9	15.1	5.1	4.2	1.4	26.6	9

CD19+		Within-tube		Between-Day		Between-Lot		Between-Replicate		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	352.7	4.7	1.3	9	2.6	14	4	6.5	1.9	26.2	7.4
2	112.9	0.1	0.1	2	1.7	1.4	1.2	4.4	3.9	8.2	7.3
3	53.7	0.8	1.5	0.5	0.8	0.7	1.3	0.7	1.4	4.4	8.2
4	106.5	2.9	2.7	1.6	1.5	2.9	2.7	3.3	3.1	10.5	9.9
5	151.2	2.4	1.6	3.6	2.4	4.6	3	2.6	1.7	10	6.6

Site to Site Reproducibility

Peripheral whole blood was collected from five healthy subjects into six TVTs from the same lot, with two TVTs from each transported at 2– 8°C prior to testing to three clinical flow cytometry laboratories. At each site, testing was performed with triplicate determinations on each of the duplicate TVTs using one lot of investigational product. For each sample, all six biomarkers were tested (CD3, CD8, CD4, CD45, CD16/CD56, CD19) using a BD FACs Canto (K040725).

CD3+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	1441.1	17.4	1.2	24.2	1.7	85.5	5.9	97	6.7
2	1435.7	13.2	0.9	28.7	2	107.4	7.5	103.9	7.2
3	372.3	9.5	2.5	20.7	5.6	12.4	3.3	28.4	7.6
4	1416.6	46.8	3.3	78.3	5.5	90.1	6.4	122.2	8.6
5	1195.1	49.9	4.2	67.1	5.6	52	4.4	86	7.2

CD8+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	592.5	26.7	4.5	8.8	1.5	48.3	8.1	54.4	9.2
2	599.2	8.5	1.4	15	2.5	54	9	52.4	8.7
3	187.3	6.5	3.5	12.2	6.5	13	6.9	21	11.2
4	868.9	23	2.6	47.7	5.5	61.2	7	89.6	10.3
5	527.1	34.9	6.6	30.1	5.7	28	5.3	51	9.7

CD4+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	782.1	4.3	0.6	17.4	2.2	26.7	3.4	48.8	6.2
2	828.2	24.7	3	12.9	1.6	46	5.6	51.6	6.2
3	165.9	0.6	0.4	7.6	4.6	5	3	16.3	9.8
4	488.2	10.9	2.2	19.5	4	23.8	4.9	42.7	8.7
5	646.1	36.4	5.6	28	4.3	13.7	2.1	48.5	7.5

CD45+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	2221.6	27.1	1.2	29.1	1.3	124.3	5.6	146.2	6.6
2	2122.5	35	1.6	47.4	2.2	167.2	7.9	162.6	7.7
3	613.3	19	3.1	38.8	6.3	22.1	3.6	53.1	8.7
4	1806.2	60.1	3.3	102.8	5.7	111.6	6.2	154.2	8.5
5	1743.8	77.4	4.4	100.6	5.8	80.2	4.6	131.6	7.5

CD16/56+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	358.1	11.2	3.1	21.9	6.1	22.9	6.4	33.5	9.4
2	484.1	37.4	7.7	28.1	5.8	36.3	7.5	57.6	11.9
3	153.5	7.6	5	10	6.5	5.2	3.4	14.7	9.6
4	275.1	14.2	5.2	19.1	6.9	12.3	4.5	29.1	10.6
5	402	16.1	4	20.3	5	26.4	6.6	34.7	8.6

CD19+		Between-tube		Between-Replicate		Between-Site		Total	
Sample	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	351.7	1.9	0.5	5.2	1.5	25.5	7.2	31.2	8.9
2	141.9	6.2	4.4	8.8	6.2	7.5	5.4	14.2	10.1
3	53.7	0.9	1.7	3.2	6	0.5	0.8	4	7.4
4	90.4	5.9	6.5	5.9	6.6	6.2	6.9	10.8	11.9
5	97.7	0.1	0.1	8.9	9.1	6.2	6.3	10.9	11.2

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Reagent stability

To evaluate the stability of unopened and opened pouches of TVTs, four peripheral whole blood samples were collected from a total of five healthy individuals. Of the four peripheral whole blood samples collected, one sample was collected into a 4 mL BD K3EDTA Vacutainer (K971449) and three samples were collected into three different lots of 3 mL TVTs, opened and unopened. The sample collected in the 4 mL BD K3EDTA Vacutainer was analyzed immediately (defined as within 6 hours of collection) for a full lymphocyte count (i.e., CD3, CD4, CD8, CD19, CD16/56 and CD45 panel) in triplicate and results were averaged. Samples taken into the TVT tubes were similarly analyzed for a full lymphocyte count in triplicate on Day 0 (<6 hours post venipuncture), and also on Days 12 and 15. The averaged absolute cell counts obtained from the 4 mL BD K3EDTA Vacutainer were considered as the control "baseline" data. Blood samples collected in TVTs were stored at 2–8°C immediately after testing on Day 0 and in between test Days 12 and 15. The results support stability of one month for both unopened and opend tube pouches. Additional testing is ongoing to support longer-term reagent stability

Stability-Vacum Stability

Stability of the TVT vacuum with regard to drawing the correct volume of blood after tubes were stored for different time periods prior to use was assessed. Three lots were tested. The volume of blood in the filled TVT tubes was measured with a 5mL serological pipette with a tolerance of +/- 0.1mL. The tube's vacuum was stable for one month. Additional testing is ongoing to support longer-term vacuum stability.

Shipping Stability

The stability of TVTs was evaluated following the transport of tubes under shipping conditions. Three unopened pouches of 3 mL TVTs, from three different lots were tested for TVT functionality, vacuum performance and qualitative stability metrics, i.e., appearance. This constituted the pre-shipping test. Following the pre-shipping test, three additional unopened pouches of 3 mL TVTs, from the same three lots used in the pre-shipping test, were packed into a cardboard box along with an electronic device for monitoring the temperature during transit. The TVTs were received two days later and shipped back to Caltag Medsystems using the same courier and method. Identical tests were conducted for TVT functionality, vacuum performance and qualitative stability metrics on these returned tubes on. This constituted the post-shipping test. The post-shipment tubes met acceptance criteria for vacuum stability and accurate measurement of absolute cells counts.

d. Detection limit:

Not applicable

e. Analytical specificity:

Peripheral blood samples were collected from healthy (n=5) and HIV positive (n=5) subjects in 4 ml K3 EDTA tubes. Following the collection the two 4 ml blood samples were split into seven 1 ml aliquots. The aliquots were spiked with various interferents listed in the table below.

Aliquot #	Interferent	Concentration
1	Control (No Interferent)	n/a
2	bilirubin	2 µg/ml (0.2 mg/dL)
3	bilirubin	200 µg/ml (20 mg/dL)
4	hemoglobin	0.025 g/ml (2.5 g/dL)
5	hemoglobin	0.035 g/ml (3.5 g/dL)
6	triglycerides	1.5 mg/ml (150 mg/dL)
7	triglycerides	5 mg/ml (500 mg/dL)

Following the addition of the interferents, each aliquot was stabilized using the appropriate amount of TransFix reagent following the manufactures instructions, i.e., 0.2 mL TransFix administered to the 1mL aliquot, and were stored at 2–8°C between test days. Aliquots were for a full lymphocyte count (i.e. CD3, CD4, CD8, CD19, CD16/56 and CD45 panel) on a BD FACS Canto II flow cytometer. Duplicate test results at each time point were used for the analysis. No interference was seen at the interferent concentration tested.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Peripheral blood samples were taken from 12 healthy subjects and 30 HIV positive subjects across three sites. At each site, whole blood samples were collected in a BD 4 mL K3 EDTA Vacutainer, a Strek Cyto Chex BCT (BCT), and a 3 ml TransFix/EDTA Vacuum Blood Collection Tubes (TVT). Samples from each tube were used to perform immunophenotyping of lymphocyte subsets using the BD Multitest kits CD3/CD16CD56/CD45/CD19 (K980858) and CD3/CD4/CD8/CD45 (K974360). The absolute counts obtained from the 4 ml K3 EDTA Vacutainter at baseline (Day 0) were used as the control. Samples from the subject and the predicate devices were tested in duplicate at at days 0, 11, and 15. Passing-Bablok regression analysis was used to anayze data.

Summary for HIV positivie subjects (n=30)				
Marker	**Time Interval**	**Intercept**	**Slope (95% CI)**	**R^2**
CD3	6 hours	-18.1	1.05 (.9973–1.142)	0.99
	11 days	-31.7	1.05 (0.9493–1.207)	0.98
	15 days	-13.8	1.04 (0.9425–1.154)	0.97
CD4	6 hours	0.6	1.02 (0.9636 –1.089)	0.99
	11 days	11.5	0.97 (0.9159–1.027)	0.99
	15 days	2.1	0.98 (0.8558–1.067)	0.98
CD8	6 hours	-60.5	1.12 (1.0481–1.196)	1
	11 days	-26.3	1.08 (0.9823–1.191)	0.98
	15 days	4.1	1.03 (0.8790 –1.179)	0.97
CD45	6 hours	-10.5	1.07 (0.9978–1.153)	0.99
	11 days	-32.5	1.05 (0.9718–1.167)	0.98
	15 days	5.3	1.05 (0.9232–1.137)	0.97
CD16/56	6 hours	1.5	1.05 (0.9278–1.172)	0.96
	11 days	10.3	0.96 (0.7929–1.118)	0.95
	15 days	15.7	0.95 (0.8079–1.139)	0.96
CD19	6 hours	3.2	1.02 (0.9361–1.078)	0.99
	11 days	-4.8	1.07 (0.9437–1.149)	0.98
	15 days	-5.8	1.05 (0.9489–1.123)	0.99

Summary for Healthy Subjects (n=12)				
Marker	**Time Interval**	**Intercept**	**Slope (95% CI)**	**R^2**
CD3	6 hours	-14.3	1.03 (0.9415–1.226)	0.99
	11 days	-178.6	1.1 (1.0392–1.445)	0.99
	15 days	-69.5	1.05 (0.9630–1.221)	0.99
CD4	6 hours	19	0.97 (0.8749–1.209)	0.99
	11 days	-76.7	1.04 (0.9407–1.212)	0.99
	15 days	-28.4	1.05 (0.9592–1.303)	0.99
CD8	6 hours	20.5	0.99 (0.5831–1.176)	0.97
	11 days	32.8	0.9 (0.4914–1.015)	0.97
	15 days	48.1	0.9 (0.4299–1.032)	0.98
CD45	6 hours	-78.1	1.05 (0.8239–1.354)	0.98
	11 days	-322.8	1.14 (0.9225–1.427)	0.97
	15 days	-42.4	1.04 (0.7490–1.293)	0.98
CD16/56	6 hours	-23	1.1 (0.6485–1.403)	0.92
	11 days	6	0.96 (0.663–1.311)	0.89
	15 days	-11.7	1 (0.7030–1.377)	0.9
CD19	6 hours	14.4	1 (0.5285–1.238)	0.98
	11 days	2.7	0.99 (0.7014–1.248)	0.98
	15 days	-5	1.08 (0.8658–1.369)	0.99

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

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

N/A

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   N/A

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.