510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
   k090810
B. Purpose for Submission:
   Clearance of a new device
C. Measurand:
   CD3, CD4, CD8, CD16, CD56, CD19, CD45 Lymphocytes
D. Type of Test:
   Quantitative and Semi-quantitative flow cytometric assay
E. Applicant:
   Microgenics Corp.
F. Proprietary and Established Names:
   Thermo Scientific Cyto-Cal™ Count Tubes
G. Regulatory Information:
   1. Regulation section:
      864.5220; Differential Cell Counter
   2. Classification:
      Class II
   3. Product code:
      GKZ; Automated differential cell counter
   4. Panel:
      Hematology (81)
H. Intended Use:
   1. Intended use(s):
      Thermo Scientific Cyto-Cal™ Count Tubes are used for determining absolute counts of leucocytes in blood.
      Thermo Scientific Cyto-Cal™ Count Tubes are used with the immunophenotyping reagents BD TriTEST™, flow cytometers BD FACS Calibur or BD FACS Canto, and software BD CellQuest or DIVA.
   2. Indication(s) for use:
      Same as Intended Use
   3. Special conditions for use statement(s):
      For Prescription Use Only
   4. Special instrument requirements:
      Thermo Scientific Cyto-Cal™ Count Tubes are for use with BD TriTEST™ reagent and the BD FACS Calibur or BD FACS Canto analyzers.
I. Device Description:
   Thermo Scientific Cyto-Cal™ Count Tubes contain uniform 5.4 um microspheres encapsulated with three proprietary oil soluble dyes. The single tube contains fluorescent beads that have equivalent emissions to multiple channels for FITC, PE, Per-CP, PE-Cy5, APC. Each tube contains a known number of fluorescent particles. Each pouch contains 25 tubes.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   BD Trucount Tubes
2. Predicate K number(s):
   K965053
3. Comparison with predicate:

Similarities		
Item	*Thermo Scientific Cyto-Cal™ Count Tubes*	**BD Trucount Tubes**
Intended Use	Thermo Scientific Cyto-Cal™ Count Tubes are used for determining absolute counts of leucocytes in blood. Thermo Scientific Cyto-Cal™ Count Tubes are used with the immunophenotyping reagents BD TriTEST™, flow cytometers BD FACS Calibur or BD FACS Canto, and software BD CellQuest or DIVA. Thermo Scientific Cyto-Cal™ Count Tubes can be used with the BD FACS™ Loader.	Used for determining absolute counts leucocytes in blood. BD Trucount Tubes are designed for use with in vitro diagnostic products such as BD TriTest reagents, and a suitably equipped flow cytometer. BD Trucount Tubes can be used with the BD FACS Loader.
Test Principle	Add the cell typing monoclonal antibody reagent and whole blood directly to the Thermo Scientific Cyto-Cal™ Count Tubes. The dried pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/μL) of positive cells in the sample can be determined by comparing cellular events to bead events. If the appropriate software, such as BD MultiSET, is used absolute counts will be determined by the software. If you are manually performing data analysis using software such as BD CellQuest, divide the number of positive cellular events, then multiply by the Thermo Scientific Cyto-Cal™ bead concentration.	Same (only with BD Trucount Tubes)
Matrix	Dried pellet of fluorescent beads in a single-use tube.	Freeze-dried pellet of fluorescent beads in a single-use tube.
Instrument	Flow cytometer	Same
Storage Condition	2 - 25°C	Same
Particle Concentration	~ 50,000/tube	Same
Lysing reagent	FACS Lyse	Same
Supplied	Each pouch contains 25 tubes, sufficient for 25 tests.	Same

K. Standard/Guidance Document Referenced (if applicable):
Not applicable.

L. Test Principle:
The test principle applies to immunophenotyping of lymphocytes. The cell typing monoclonal antibody reagent and whole blood are directly added to the Thermo

Scientific Cyto-Cal™ Count Tubes. The dried pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/µL) of positive cells in the sample can be determined by comparing cellular events to bead events. If the appropriate software, such as BD MultiSET, is used absolute counts will be determined by the software. If performing manual data analysis using software such as BD CellQuest, divide the number of positive cellular events, then multiply by the Thermo Scientific Cyto-Cal™ bead concentration.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*

   Precision studies were performed to assess absolute count within-run reproducibility for low, normal, and high CD3 and CD3CD4 samples. For each sample, twenty-one (21) aliquots of whole blood were stained with CD3/CD4/CD45 TriTEST reagent using Thermo Scientific Cyto-Cal™ Count Tubes. The range of CVs for CD3 cells, observed for all samples were 4.0% - 6.2%. **Acceptance Criteria**: Medium and High Cell Concentration Samples - $\leq$ 5% CV, Low Cell Concentration Samples (Semi-quantitative with-in run) - 7.5 % CV. The range of CD3CD4 cells, observed for all samples were 4.3% - 6.9%. Precision data met the acceptance criteria.

   Within-run results:

Sample	N	Subset	Mean cells/µL	CV%
Low	21	$CD3^+$	549	6.2
		$CD3^+CD4^+$	369	6.9
Medium	21	$CD3^+$	1875	3.8
		$CD3^+CD4^+$	1220	3.9
High	21	$CD3^+$	3602	4.9
		$CD3^+CD4^+$	1996	4.7

   b. *Linearity/assay reportable range:*

   To assess linearity and recovery, a dilution recovery study was performed. One fresh whole blood sample was concentrated by removing plasma. This high sample was diluted with serum to 80%, 60% 40%, 20% and 0%. The results are as follows:

   Expected results vs. measured values

Subset	r	Slope	Intercept
$CD3^+$	0.9996	0.991	17.492
$CD3^+CD4^+$	0.9990	1.0094	12.159

   Recovery was within 10% of expected value for levels tested.

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

   A reagent Shelf-Life Stability study was performed on three lots of Cyto-Cal™ Count Tubes at room temperature (20°-25°C). Bead counts were measured from Day 0 to 16 months and recovery was expected to be within

±10% of Day 0 value for up to 16 months. Recovery was greater than 98% of Day 0 for all three lots for up to 16 months.

    d. *Detection limit:*
        Not applicable.

    e. *Analytical specificity:*
An interference study was performed to determine the potential of endogenous and exogenous substances to affect accuracy. The interference was assessed by adding known amounts of interfering substances into whole blood with a CD4+ concentration of 398 cells/µL. **Acceptance Criteria:** CD4+ Count Measured recovers within ± 10% of CD4$^+$. No interference was observed for samples adjusted in the pH range of 6.8 – 8.8, in addition to sample conditions listed below:

EDTA: No significant interference from EDTA 10 mg/mL
Icterus (jaundice): No significant interference from Bilirubin up to 20 mg/dL
Hemoglobin: No significant interference from Hemoglobin up to 20 g/dL
Lipemia: No significant interference from Triglycerides up to 500 mg/dL
Total Protein: No significant interference from Albumin up to 12 g/dL
Package insert lists other substances tested with specific concentrations.

    f. *Assay cut-off:*
        Not applicable.

2. Comparison studies:

    a. *Method comparison with predicate device:*
Method comparison studies were performed at one internal site (70 samples) and two external sites (40 samples). The results are as follows:

Internal Site

Subset	n	r	Slope	Intercept
CD3$^+$	70	0.9969	0.996	-0.3
CD4$^+$	70	0.9956	0.995	-3.6

External Sites combined

Subset	n	r	Slope	Intercept
CD3$^+$	40	0.9769	0.967	-29.4
CD4$^+$	40	0.9847	1.021	-29.1
CD8$^+$	40	0.9646	0.911	-7.1
CD16$^+$ CD56$^+$	20	0.9916	1.010	-7.7
CD19$^+$	20	0.9931	0.917	-0.36
CD4/8 ratio	40	0.9942	0.967	0.064
CD45$^+$	20	0.9812	1.016	-109.6

    b. *Matrix comparison:*
        Not applicable.

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):
          Not applicable.
    4. <u>Clinical cut-off</u>**:**
         Not applicable.
    5. <u>Expected values/Reference range:</u>
       Reference ranges as published by the University of California, San Francisco:
       CD3 (T) Cells: 690 – 2540 cells/µL
       CD4 (helper – inducer) T Cells: 410 – 1590 cells/µL
       CD8 (cytotoxic – suppressor) T Cells: 190 – 1140 cells/µL
       CD4/8 (H/S) Ratio: 0.8 – 4.2
       CD19 (B) Cells: 90 – 660 cells/µL
       CD16, CD56 Natural Killer (NK) Cells: 90 – 590 cells/µL

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.