**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

**A. 510(k) Number:**

K053497

**B. Purpose for Submission:**

New Device

**C. Measurand:**

CD4 T-Lymphocytes

**D. Type of Test:**

Quantitative, Flow cytometry

**E. Applicant:**

Guava Technologies, Inc.

**F. Proprietary and Established Names:**

Guava EZCD4 System

**G. Regulatory Information:**

1. Regulation section:

   864.5220, Automated Differential Cell Counter

2. Classification:

   Class II

3. Product code:

   GKZ, Counter, Differential Cell

4. Panel:

   Hematology (81)

## H. Intended Use:

1. Intended use(s):

   The EZCD4 Assay is intended to be performed on a Guava PCA System with CytoSoft 2.3 version software which includes three modules; EZCD4, Guava Check and Clean and Shutdown. The system is intended to identify and quantify the absolute counts of CD4 T-Lymphocytes in EDTA whole blood. The GuavaEZCD4 system is intended for the ongoing monitoring of patients with documented diagnosis of an immunodeficiency disease. The Guava EZCD4 system is intended for use only by trained laboratory professionals.

2. Indication(s) for use:

   The EZCD4 Assay is intended to be performed on a Guava PCA System with CytoSoft 2.3 version software which includes three modules; EZCD4, Guava Check and Clean and Shutdown. The system is intended to identify and quantify the absolute counts of CD4 T-Lymphocytes in EDTA whole blood. The GuavaEZCD4 system is intended for the ongoing monitoring of patients with documented diagnosis of an immunodeficiency disease. The Guava EZCD4 system is intended for use only by trained laboratory professionals.

3. Special conditions for use statement(s):

   Not applicable.

4. Special instrument requirements:

   Not applicable.

## I. Device Description:

The Guava EZCD4 System is an optimized cell analysis system consisting of the Guava PCA instrument, CytoSoft software for data acquisition and analysis and an EZCD4 Reagent Kit consisting of optimized reagent and protocols. The Guava PCA instrument incorporates a new technology termed micro capillary cytometry. The device is a 3-parameter flow cytometer that utilizes a solid state 532 nm green laser, a fixed optical and fluidic system, a self-aligning flow cell and a micro capillary delivery system to perform cell analysis. The micro-syringe stepper pump allows the use of small sample volumes and volumetric measurement for the determination of absolute counts per microliter. The instrument includes a laptop computer. The Guava EZCD4 Reagent Kit is a two-color direct immunofluorescence kit used for the enumeration of mature CD4+ T lymphocytes in human blood. It consists of a

monoclonal anti-human CD3 antibody conjugated to the tandem dye, phycoerythrin (PE)-Cy5 (PECy5) and a monoclonal anti-human CD4 antibody conjugated to PE.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   Becton Dickenson MultiTest CD3/CD8/CD45/CD4
   Becton Dickenson TriTest CD3 FITC/CD4 PE/CD45 PerCP

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

   K974360, K965053

3. Comparison with predicate:

| **Similarities** | | | |
|---|---|---|---|
| **Item** | ***Guava EZCD4*** | ***BD MultiTest*** | ***BD TriTest*** |
| Intended Use | Absolute counts of CD4 T-Lymphocytes | Percentages and absolute counts of mature human T lymphocytes (CD3+), suppressor; cytotoix (CD3+CD8+) T-lymphocyte subsets, and helper/inducer (CD3+CD4+) T-lymphocyte subsets | Identifying and enumerating percentages of T lymphocytes (CD3+), and helper (CD3+CD4+) cells |
| Specimen | EDTA Whole Blood | Same | Same |
| Data Acquisition | The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information on the relative cell size and relative fluorescence intensity. | The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information on the cell's size, internal complexity and relative fluorescence intensity. | The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information on the cell's size, internal complexity and relative fluorescence intensity. |
| Reagent Storage | Antibody reagents: 2 to 8°C<br>Lyse and Fixative: 18 to 25°C | 2 to 8°C | 2 to 8°C |
| Specimen Handling | Staining within 72 hours of draw and analyzed within 24 hours of staining. | Staining within 48 hours of draw and analyzed within 24 hours of staining. | Staining within 48 hours of draw and analyzed within 24 hours of staining. |

| Differences | | | |
|---|---|---|---|
| Item | ***Guava EZCD4*** | ***BD MultiTest*** | ***BD TriTest*** |
| Laser | 532 nm diode | 635nm and 488nm | 635nm and 488nm |
| Instrument | PCA | FACSCalibur | FACSCalibur |
| Analysis | Two color immunofluorescence reagents | Four color fluorochrome labeled reagents | Three color fluorochrome labeled antibodies |
| Antibodies | Anti-human CD4-PE reagent (clone EDU-2) and anti-human CD3PECy5 reagent (clone UCHT-1) | FITC-labeled anti-CD3 antibody, clone SK7; PE-labeled anti-CD8 antibody, clone SK1; PERCP-labeled anti-CD45 antibody, clone 2D1 (HLe-1) and APC-labeled anti-CD4 antibody clone SK3 | FITC-labeled anti-CD3 antibody, clone SK7; PE-labeled anti-CD8 antibody, clone SK1; PERCP-labeled anti-CD45 antibody, clone 2D1 (HLe-1) and PE-labeled anti-CD4 antibody clone SK3. |

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

*Class II Special Control Guidance Document: Premarket Notification for Automated Differential Cell Counters for Immature or Abnormal Blood Cells Final Guidance for Industry and FDA, December 4, 2001*

## L. Test Principle:

The Guava EZCD4 Kit is a two-color direct immunofluorescence reagent kit for enumeration of mature CD4+ T lymphocytes in human blood. The kit consists of a monoclonal anti-human CD3 antibody conjugated to the tandem dye phycoerythrin (PE)-Cy5 (PECy5), a monoclonal anti-human CD4 antibody conjugated to PE, Guava 1X Lysing Solution to lyse erythrocytes and Guava Fixative to preserve the cells. The CD3 antibody uniquely identifies T cells and recognizes an epitope that is expressed on the epsilon chain of the CD3/T cell antigen receptor (TcR) complex. The CD4 antibody allows the identification of human helper/inducer CD4+T cell (HLA Class II reactive) and recognizes a 60,000 Da MW surface antigen. CD4 is also present on monocytes but at much lower density and lack co-expression of the CD3 molecule. The prepared specimen is acquired by the PCA instrument. The cells travel past a laser beam and scatter the laser light. The stained cells fluoresce and both the scatter and fluorescence signals are detected by the instrument and analyzed to give the final result.

## M. Performance Characteristics (if/when applicable):

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

   *a. Precision/Reproducibility:*

   Intra-Laboratory reproducibility was established by testing at four independent geographically distributed sites in the US. At each site, 10 replicate whole blood samples were analyzed from each of 3 abnormal donors representing each of three

EZCD4+ absolute count ranges. These ranges were Low range (0-200), Mid range (201-500) and High range (501-2000). Means, standard deviations (SD) and coefficients of variation (CV) were determined for each site in each range.

The results are as follows (Intra-laboratory Reproducibility):

| Study Site | Range | Mean EZCD4 CD4+ T Cells/µL | SD | CV | n |
|---|---|---|---|---|---|
| 1 | Low | 178.41 | 24.08 | 13.50 | 10 |
| | Mid | 494.97 | 39.89 | 8.06 | 10 |
| | High | 676.45 | 32.16 | 4.75 | 10 |
| | | | | | |
| 2 | Low | 72.61 | 7.59 | 10.45 | 10 |
| | Mid | 417.27 | 30.06 | 7.20 | 10 |
| | High | 655.72 | 32.29 | 4.92 | 10 |
| | | | | | |
| 3 | Low | 81.82 | 9.04 | 11.05 | 10 |
| | Mid | 366.36 | 14.17 | 3.87 | 10 |
| | High | 870.43 | 30.99 | 3.56 | 10 |
| | | | | | |
| 4 | Low | 165.44 | 7.76 | 4.69 | 10 |
| | Mid | 373.20 | 13.79 | 3.69 | 10 |
| | High | 559.65 | 18.07 | 3.23 | 10 |

*b. Linearity/assay reportable range:*

In the linearity study, Expected versus Observed values of absolute CD4 T cell counts were determined by the preparation of a series of blood cell aliquots, each aliquot consisting of a decreasing volume of a bulk blood sample of known "high range absolute CD$+T cell count and an increasing volume of a bulk blood sample of known "low range" absolute CD4+ T cell counts. All cell aliquots were prepared in duplicate and a total of 22 aliquots (11 pairs) were prepared.

The linear regression analysis results are as follows:

$y = 1.011x - 45.237 \quad r^2 = 0.9866$

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

Open vial stability testing was performed and determined to be up to five weeks when stored at $2^o$ to $8^o$ C.

*d. Detection limit:*

Special indications were included in the linearity study protocol for the concentration if absolute CD4+ T cells required of the "low bulk blood sample (<50 CD4+ T cells/µL) and of the "high range" bulk blood sample (>2000 CD4+ T cell/µL), to

enable the dynamic range of the Guava EZCD4 System to be demonstrated. The dynamic range is determined to be 50 to 2500 CD4+ T cells/ µL.

*e. Analytical specificity:*

Not applicable.

*f. Assay cut-off:*

Not applicable.

2. Comparison studies:

*a. Method comparison with predicate device:*

Comparison studies were performed at four geographically diverse clinical sites. A total of 365 abnormal donors in three CD4+ absolute count ranges were collected. These included approximately 30 donors with count within the low range (0-200), 30 within the mid range (201-500) and 30 within high range (501-2000) samples at each site. Results are as follow:

Regression analysis

| Study Site | n | R squared | Slope | Intercept | Range |
|---|---|---|---|---|---|
| 1 | 92 | 0.95 | + 1.00 | 18.64 | 13-1465 |
| 2 | 91 | 0.93 | + 0.96 | 35.51 | 17-1175 |
| 3 | 88 | 0.98 | + 1.17 | 18.46 | 47-1439 |
| 4 | 94 | 0.98 | + 0.95 | 13.29 | 8-1076 |

*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. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Laboratories must establish their own normal reference range. Reference ranges provided are for information only.

**N. Instrument Name:**

Guava Technologies, Inc, Guava PCA

**O. System Descriptions:**

1. Modes of Operation:

Random access

2. Software:

The Guava PCA CytoSoft 2.3 version software includes three modules: EZCD4, Guava Check and Clean and Shutdown. The EZCD4 is designed to acquire and analyze data specifically for CD4+T cells results. Guava Check controls the instrument for purposes of determining if the system is functioning properly. Clean and Shutdown prepares the instrument for daily shutdown.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No_____

3. Specimen Identification:

Instrument auto numbering

4. Specimen Sampling and Handling:

Whole blood is collected aseptically by venipuncture into a sterile EDTA (lavender top) blood collection tube. Blood should be stained within 72 hours of collection for optimal results. Unstained anticoagulated blood should be maintained at 18 to 25°C prior to sample processing. Samples should be acquired within 24 hours of staining.

5. Calibration:

   The Guava Check Kit is used daily to verify the performance by assessing counting accuracy and fluorescence detection using a standard fluorescence bead reagent.

6. Quality Control:

   Commercially available whole blood control should be run daily to optimize instrument settings and as a quality control check of the system

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:**

**Q. Proposed Labeling:**

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

**R. Conclusion:**

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