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Date: August 16, 1996

Title: Summary of Safety and Effectiveness Information For 510(k) Premarket Notification

Product: tetraONE™ SYSTEM for EPICS® XL Flow Cytometry Systems and CYTO-STAT® tetraCHROME™ CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 Monoclonal Antibody Reagent

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Common or Usual or Classification Name: Lymphocyte Immunophenotyping System with Reagents and Software for Flow Cytometry

Product Classification: Product Code: GKZ; C.F.R. Section: 864.5220; Classification Panel: Hematology and Pathology Devices; Device Class: II

Intended Use: The tetraONE™ SYSTEM for EPICS® XL Flow Cytometry Systems and CYTO-STAT® tetraCHROME™ CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 Monoclonal Antibody Reagent combines a four-color fluorescent monoclonal antibody reagent, four quality control reagents, an optional absolute count reagent, and software for automated analysis of lymphocyte populations in whole blood using EPICS® XL Flow Cytometry Systems with SYSTEM II™ Software. The system is intended "For In Vitro Diagnostic Use" and allows simultaneous identification and enumeration of total CD4+, total CD8+, total CD3+, dual-positive CD3+/CD4+ and dual-positive CD3+/CD8+ T lymphocytes percentages and absolute counts. The system also provides the T4/T8 ratio.

Substantial Equivalence: *510(k) Premarket Notification:* *K922745*
CYTO-STAT®/COULTER CLONE® CD3(IgG1)-FITC/T4-RD1 Monoclonal Antibody Reagent
510(k) Premarket Notification: *K922744*
CYTO-STAT®/COULTER CLONE® CD3(IgG1)-FITC/T8-RD1 Monoclonal Antibody Reagent

Product Differences: CD45/CD4/CD8/CD3, CD3/T4 and CD3/T8 are essentially identical with respect to features and principles of operation. The new and comparator systems use the same, well-established, state-of-the-art technologies of immunophenotyping with monoclonal antibodies and flow cytometry to measure cellular components in whole blood via immunofluorescence analysis. Further, the intended use of the new and comparator systems is the *same*. Also, each liquid reagent allows simultaneous identification and enumeration of more than one lymphocyte population in a single specimen using a single reagent.

The new and comparator systems differ in only a few respects. One difference results from the more advanced software for the new tetraONE™ SYSTEM. The tetraONE™ SYSTEM software is designed to further simplify flow cytometric analysis by increasing automated modes of operation and the accuracy, precision and reliability of results. The new and comparator systems also differ in that the new tetraONE™ SYSTEM does not require an isotypic control for monitoring and adjusting for non-specific and non-targeted monoclonal antibody binding to irrelevant cellular populations. The tetraONE™ SYSTEM software monitors and adjusts for such binding by automatically placing cursors based on the separation of positive and negative peaks.

There are two differences between the reagents.

a. **Lymphocyte Gating:** The CD45/CD4/CD8/CD3 reagent contains a monoclonal antibody, CD45, to identify a lymphocyte gate to allow CD3+, CD4+, CD8+, dual-positive CD3+/CD4+ and dual-positive CD3+/CD8+ measurements. In contrast, CD3/T4 and CD3/T8 require a separate reagent, Mo2-RD1/KC56 (T-200)-FITC, for this purpose.

b. **Fluorescent Labeling:**
CD45/CD4-RD1/CD8-ECD/CD3-PC5:
CD45: FITC (Fluorescein Isothiocyanate); CD4: RD1 (Phycoerythrin); CD8: ECD (Phycoerythrin-Texas Red); CD3: PC5 (Phycoerythrin-Cy5).

CD3/T4 and CD3/T8:
CD3: FITC (Fluorescein Isothiocyanate); T4: RD1; T8: RD1 (Phycoerythrin).

Product Testing: Product testing to assess the performance of CD45/CD4/CD8/CD3 is described below. Studies were designed in line with instructions for use given in the tetraONE™ SYSTEM Guide, Package Inserts, Product Manuals, and performance specifications. Specimens were assayed with CD3/T4 and CD3/CD8 for comparison purposes. The results of product testing demonstrated that CD45/CD4/CD8/CD3 met all performance specifications and provided mature T (CD3+), inducer T (CD4+; CD3+/CD4+) and suppressor/cytotoxic T (CD8+; CD3+/CD8+) lymphocyte values comparable to those of CD3/T4 and CD3/T8.

1. Accuracy:
Normal and abnormal (e.g., Human Immunodeficiency Virus, organ transplant, autoimmune disease, low white blood cell count) whole blood specimens were collected from geographically diverse populations of males and females unselected as to race and ranging in age from 18 to 85 years. Specimens were divided, processed as lysed preparations and assayed in parallel with CD45/CD4/CD8/CD3, CD3/T4 and CD3/T8. The CD3+, CD4+, CD8+, CD3+/CD4+ and CD3+/CD8+ percentages expressed in terms of the total lymphocyte count and absolute counts (cells/μL) were determined with EPICS® XL-MCL flow cytometers gated on lymphocytes. White blood cell counts and 5-part differentials were obtained for all specimens. CD3/T4 and CD3/T8 values were corrected for lymphocyte purity (Lymphocyte Gate Limits: lymphocyte recovery ≥ 90%; lymphocyte purity ≥ 85%).

   Results analyzed in terms of minimums, maximums, means ± 1 SD, confidence intervals with 95% limits, regression and correlation analyses, and analyses of variance demonstrated that CD45/CD4/CD8/CD3, CD3/T4 and CD3/T8 identify and enumerate essentially identical numbers of the targeted lymphocytes in whole blood specimens.

2. Linearity:
Three replicate measurements were made on a concentrated COULTER™ CYTO-TROL™ Control Cells sample serially diluted to achieve a range of CD3+, CD4+ (CD3+/CD4+) and CD8+ (CD3+/CD8+) lymphocyte concentrations. Samples were assayed with CD45/CD4/CD8/CD3 and analyzed on an EPICS® XL-MCL flow cytometer gated on lymphocytes. Values were expressed in terms of absolute counts (cells/μL).

   Results analyzed in terms of regression and correlation analyses for recovered versus expected absolute counts demonstrated linearity of the assay.

3. Within Run (Intralaboratory) Precision:
Ten replicate measurements were made for each of three levels of CD3+, CD4+ (CD3+/CD4+) and CD8+ (CD3+/CD8+) lymphocyte concentrations using a COULTER EPICS® XL-MCL flow cytometer gated on lymphocytes. Levels were obtained by selective depletions of a normal whole blood specimen and assayed with CD45/CD4/CD8/CD3. Values were expressed in terms of % of the total lymphocyte count.

Results analyzed in terms of mean ± 1 SD and CV demonstrated Within Run (Intralaboratory) Precision of the assay.

4. Interlaboratory Precision:
Ten replicate measurements were made on the same day using different laboratories and EPICS® XL-MCL flow cytometers. All measurements were made on a single normal whole blood specimen divided and assayed with CD45/CD4/CD8/CD3. Values were expressed in terms of % of the total lymphocyte count.

Results analyzed in terms of mean ± 1 SD and CV demonstrated Interlaboratory Precision of the assay.

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August 16, 1996

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