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Date: October 11, 1996

Title: Summary of Safety and Effectiveness Information for
"K950742 510(k) Additional Information"

Product:

CYTO-STAT® triCHROME™ CD8-FITC/CD4-RD1/CD3-PC5 Monoclonal Antibody Reagent
with
CYTO-STAT® triCHROME™ MsIgG1-FITC/MsIgG1-RD1/MsIgG1-PC5 Isotypic Control

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Common or Usual or Classification Name: Lymphocyte Immunophenotyping Monoclonal
Antibody Reagent and Isotypic Control

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

Intended Use: CYTO-STAT® triCHROME™ CD8-FITC/CD4-RD1/CD3-PC5 Monoclonal Antibody
Reagent is a three-color fluorescent reagent comprised of three murine monoclonal
antibodies. Each antibody is labeled with a different color fluorochrome. The reagent
allows simultaneous identification and enumeration of total CD3+, total CD4+, total
CD8+, dual CD3+/CD4+ and dual CD3+/CD8+ lymphocytes in whole blood by flow
cytometry. An isotypic control, CYTO-STAT® triCHROME™ MsIgG1-
FITC/MsIgG1-RD1/MsIgG1-PC5, is used to monitor nonspecific staining.

CYTO-STAT® triCHROME™ MsIgG1-FITC/MsIgG1-RD1/MsIgG1-PC5 Isotypic
Control is a three-color fluorescent reagent comprised of three murine monoclonal
antibodies. Each antibody is labeled with a different color fluorochrome. This product
is intended for use as a quality control reagent to monitor the levels of nonspecific
antibody binding in cell surface staining procedures which use CYTO-STAT®
triCHROME™ CD8-FITC/CD4-RD1/CD3-PC5 Monoclonal Antibody Reagent to
identify and enumerate total CD3+, total CD4+, total CD8+, dual CD3+/CD4+ and
dual CD3+/CD8+ lymphocytes in whole blood by flow cytometry.

Substantial Equivalence: "K950742 510(k) Premarket Notification"

CYTO-STAT®/COULTER CLONE® CD3-ECD/T4-RD1/T8-FITC Monoclonal Antibody Reagent
with
CYTO-STAT®/COULTER CLONE® MsIgG2b-ECD/MsIgG1-RD1/MsIgG1-FITC Isotypic Control

Product Differences: CD8/CD4/CD3 with MsIgG1/MsIgG1/MsIgG1 and CD3/T4/T8 with MsIgG2b/MsIgG1/MsIgG1 are essentially identical with respect to features and principles of operation. Both the *revised* and *original* product 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 each system is the *same*. The liquid monoclonal antibody reagents allow simultaneous identification and enumeration of more than one lymphocyte population (total CD3+, total CD4+, total CD8+, dual CD3+/CD4+ and dual CD3+/CD8+) in a single specimen using a single reagent. Each system also uses separate reagents to 1) identify a lymphocyte gate (i.e., CYTO-STAT®/COULTER CLONE® Mo2-RD1/KC56 (T-200)-FITC); and 2) monitor nonspecific binding (i.e., isotypic control).

The few differences (*italicized*) between the reagent and isotypic control formulations are as follows:

- | | | |
|------------------------------|---|---|
| 1. CD3 Clone: | CD8/CD4/CD3:
CD3/T4/T8: | <i>UCHT1</i>
<i>HIT3a</i> |
| 2. CD3 Isotype: | CD8/CD4/CD3:
CD3/T4/T8: | <i>MsIgG1</i>
<i>MsIgG2a</i> |
| 3. Isotypic Control Clone: | MsIgG1/MsIgG1/MsIgG1: -----;
MsIgG2b/MsIgG1/MsIgG1: <i>MsIgG2b=MPC11</i> ; | <i>MsIgG1=2T8-2F5</i>
<i>MsIgG1=2T8-2F5</i> |
| 4. Isotypic Control Isotype: | MsIgG1/MsIgG1/MsIgG1: -----;
MsIgG2b/MsIgG1/MsIgG1: <i>MsIgGb</i> ; | <i>MsIgG1</i>
<i>MsIgG1</i> |
| 5. CD3 Fluorochrome: | CD8/CD4/CD3:
CD3/T4/T8: | <i>PC5 (Phycoerythrin-Cy5)</i>
<i>ECD (Energy-Coupled Dye)</i> |

Product Testing: Product testing to assess the performance of the CD8/CD4/CD3 product system is described below. Studies were designed in line with instructions for use given in the Package Inserts, Product Manuals, and performance specifications. Specimens were assayed with the CD3/T4/T8 product system for comparison purposes. The results of product testing demonstrated that CD8/CD4/CD3 met all performance specifications and provided mature T (CD3+), inducer T (CD4+; dual CD3+/CD4+) and suppressor/cytotoxic T (CD8+; dual CD3+/CD8+) T lymphocyte values comparable to those of CD3/T4/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 CD8/CD4/CD3 and CD3/T4/T8. The CD3+, CD4+, CD8+, CD3+/CD4+ and CD3+/CD8+ percentages expressed in terms of the total lymphocyte count and absolute counts (cells/ μ L) were determined with a COULTER® EPICS® XL-MCL flow cytometer gated on lymphocytes. White blood cell counts and 5-part differentials were obtained for all specimens using the COULTER® STKS. Absolute counts were determined using both the Standard (Indirect) Method, and the Flow-Count (Direct) Method with Flow-Count™ Fluorospheres. All values were corrected for lymphocyte purity (Lymphocyte Gate Limits: lymphocyte recovery \geq 90%; lymphocyte purity \geq 85%).

Results analyzed in terms of minimums, maximums, means \pm 1 SD, confidence intervals with 95% limits, regression and correlation analyses, and analyses of variance, demonstrated that CD8/CD4/CD3 and CD3/T4/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 CD8/CD4/CD3 and analyzed on a COULTER® 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 CD8/CD4/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.

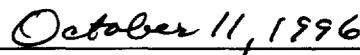
4. **Interlaboratory Precision:**

Ten replicate measurements were made on the same day using different laboratories and COULTER® EPICS® XL-MCL flow cytometers. All measurements were made on a single normal whole blood specimen divided and assayed with CD8/CD4/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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Date