Memorandum

Date September 26, 1991

From Chief, Hematology/Pathology Branch, Division of Clinical Laboratory Devices (HFZ-440), Office of Device Evaluation, Center for Devices and Radiological Health

Subject

Draft Guidance for 510(k) Submission of Lymphocyte Immunophenotyping Monoclonal Antibodies

To

Interested Manufacturers

We have developed a draft document entitled, "Guidance Document for 510(k) Submission of Lymphocyte Immunophenotyping Monoclonal Antibodies." Since the document lists items we will be reviewing, it is intended to assist manufacturers in the preparation of marketing submissions for these types of devices.

As this area of in vitro diagnostics is rapidly expanding in the clinical laboratory, we are soliciting your ideas, recommendations, and comments regarding the enclosed draft guidance document. We will appreciate receiving your comments so we can incorporate as many improvements as possible in a revision. Additional copies of this document may be obtained through the Office of Small Manufacturers Assistance from Geoffrey Clark, (301) 443-6597.

Please address comments to:

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DRAFT: Guidance Document For 510(k) Submission of Lymphocyte
Immunophenotyping Monoclonal Antibodies.

This is a flexible document representing the current major concerns and
suggestions regarding lymphocyte immunophenotyping in vitro diagnostic
devices employing monoclonal antibodies methodology with flow
cytometry, fluorescence and light microscopy.   It is based on 1)
current basic science, 2) clinical experience and 3) previous
submissions by manufacturers to the FDA.   As advances are made in
science and medicine, these review criteria will be re-evaluated and
revised as necessary to accommodate new knowledge.

REVIEW CRITERIA FOR ASSESSMENT OF LYMPHOCYTE IMMUNOPHENOTYPING IN VITRO DIAGNOSTIC DEVICES USING MONOCLONAL ANTIBODIES WITH FLOW CYTOMETRY, FLUORESCENCE AND LIGHT MICROSCOPY

For the purposes of this guidance document lymphocytes are defined as
those lymphocytes possessing the cluster of differentiation (CD)2, CD3,
CD4, CD5, CD8, CD16, CD19, CD20, and CD56.   This document also covers
CD45, CD14, and CD13 which are used for lymphocyte gating and HLA-DR
used for the detection of activated CD3 positive cells.   This document
also covers anti-isotype controls, and secondary antibodies labeled
with biotin or a chromogen (any compound which is not a dye but is
capable of becoming one).   In this context, fluorescein isothiocyanate
(FITC), phycoerythrin (PE), other fluorochromes, and horseradish
peroxidase, etc. are chromogens.

PRODUCT CODE: GKZ

REGULATION NUMBER: 21 CFR § 864.5220

PANEL: Hematology/Pathology

CLASS: II

REVIEW REQUIRED: 510(k)

DEFINITION: This generic type device is intended for use in clinical
laboratories as an in vitro test for the quantitative measurement of
lymphocytes and their subsets using monoclonal antibodies (MAb) by flow
cytometry, light and fluorescence microscopy.

PURPOSE: The purpose of this document is to provide guidance on
information to present to the Food and Drug Administration (FDA) before
a device utilizing MAbs to immunophenotype lymphocytes may be cleared
for marketing.   This information enables FDA to make better informed
decisions based on a uniform data base.

Where applicable this document references sections from the National
Committee for Clinical Laboratory Standards (NCCLS) Clinical
Applications of Flow Cytometry: Quality Assurance and
Immunophenotyping of Peripheral Blood Lymphocytes.[1]   If not otherwise
defined, the definitions for various terms used in this document are
taken from the NCCLS document.

I.   Background.
      Current clinical use of the above MAbs is for enumeration
      (percentage) of the CD either as a single subset or a group
      within a subset using multi-parameter and multi-color
      methodology. Immunophenotyping lymphocytes with MAbs can be used
      as an aid in the determination of the immune status. Much work
      has been done with CD4 and CD8 values in their relation with HIV
      (human immunodeficiency virus) infection and the progression of
      acquired immune deficiency syndrome (AIDS) and other immune
      deficiency diseases. Enumerating total CD4 positive and CD8
      positive lymphocytes in HIV infected individuals allows the
      physician to place these individuals into various staging
      groups.[2,3] The clinical significance of lymphocyte
      immunophenotyping MAbs has been shown for this situation. At
      present, the only intended use for which lymphocyte
      immunophenotyping MAbs are cleared is the enumeration of
      lymphocyte subsets. The clinical significance of any subset
      associated with leukemias and lymphomas has yet to be universally
      accepted. A change of the intended use of the above referenced
      MAbs from enumeration of normal lymphocyte subsets in human blood
      to a more specific intended use of typing various leukemias and
      lymphomas would require a premarket application for approval to
      market (PMA). This would be considered a new intended use for
      these devices and would require a more rigorous process for
      approval. The clinical significance for MAbs other than the
      above uses has not been shown or has not been widely accepted.
      The PMA procedure may also include MAbs for activation markers
      (other than CD3+/HLA-DR), platelets, other leukocytes, and
      precursor human blood cells.

      Exceptions to the above are the MAbs which recognize CD45, CD13,
      CD14 and keyhole limpet hemocyanin used for anti-isotype
      controls. Although, some clinical relevance may be placed on the
      variation of these CDs in a patient, their primary uses at this
      time are quality control (QC) and as an aid in gating of samples
      on a flow cytometer. An intended use for enumeration of
      lymphocytes for these MAbs is not appropriate at this time.

II.   Device Description.
      Monoclonal antibodies are directed against specific CD epitopes
      on human lymphocytes. The MAbs may be used alone or in
      conjunction with each other, labeled or unlabeled. The MAbs are
      either labeled (having a chromogen or avidin attached) or
      unlabeled (to be used with a secondary antibody labeled with an
      appropriate chromogen). The use of a biotin-avidin complex and
      labeled secondary antibody is normally used for amplification of
      a weak signal. More than one MAb may be present to enumerate
      specific groups within a subset of lymphocytes, such as,
      CD8+/CD11b+, CD8+/CD11b-, CD8-/CD11b+, and CD8-/CD11b- cells.
      Additionally, three chromogens may be used to further enumerate
      subsets, such as CD8+/CD11+/CDx+.

      A.   Principle of the Test.
            A specific MAb is added to a sample of whole blood or

isolated mononuclear cells. After an appropriate incubation
period and washes, the lymphocytes which have been labeled
with the antibody/chromogen complex are enumerated.
Depending upon the method used (cytometry or microscopy), the
chromogen is detected with light excitation or chemical color
change.

B. Specimen Type(s).
Anticoagulated whole blood; Lymphocytes are isolated from
erythrocytes by accepted methods such as whole blood lysis or
gradient centrifugation. Anticoagulants encompass all of the
acceptable anticoagulants used for specimen collection of
whole blood for lymphocyte immunophenotyping. NCCLS lists
the following anticoagulants; ethylenediaminetetraacetic acid
(EDTA), heparin, and acid citrate dextrose (ACD).[1] If a
manufacturer wishes to make a claim for an anticoagulant
other than the above, comparison data is needed for the new
anticoagulant with the anticoagulants listed above.
Lymphocytes are either fixed (labeled samples suspended in
buffered formaldehyde, pH 7.2 or paraformaldehyde), or
unfixed and contain azide.

III. Specific Performance Characteristics.
The FDA requires different types and amounts of data and
statistical analyses to be included in a manufacturer's
application for marketing of in vitro diagnostic devices.
Additional data may be necessary to substantiate certain claims
of intended use or clinical significance. If data presented in
the submission adequately demonstrates there is no significant
difference in test results between different sample
types/matrices using appropriate statistical studies; e.g.,
regression studies or paired T tests, then test performance
characteristics may be determined with one specimen of choice.
Otherwise, performance characteristics must be demonstrated for
all sample types/matrices claimed for use or which demonstrate
statistical differences.

In addition to proof that the MAb clone being submitted has been
classified by the World Health Organizations' International
Workshops on Human Leucocyte Differentiation Antigens, the
submission of the following data is required to determine
substantial equivalence of MAbs for immunophenotyping of
lymphocytes using flow cytometry, fluorescence, or light
microscopy:

A. Analytical/Laboratory/In Vitro Studies.
Specific parameters of importance to the use of the MAb
should be supported by data determined with that MAb clone.
It should be demonstrated that the performance of the MAb is
substantially equivalent to another legally marketed device.
Present test data with analyses and conclusions. The data and
statistical evaluation should be sufficient to determine if
the device is substantially equivalent and/or safe and
effective for all claimed specimen type(s)/matrices.

Address the following performance characteristics in a
submission for lymphocyte immunophenotyping using flow
cytometry or fluorescence/light microscopy:

1. Performance Characteristics.

    a) Antibody Specificity (For Immunological Assays).
        Investigate the cross-reactivity and interference of
        any potentially interfering substances using the
        assay system. Submit data documenting any
        crossreactivity of the antibodies with the following
        cell populations:
        *   monocytes
        *   granulocytes
        *   platelets
        *   red blood cells

        Additional data should be submitted to demonstrate
        that MAbs in combination are equivalent to the single
        MAbs alone.

    b) Linear Range.
        Determine the linear range of the assay using 4 each
        low, normal, and abnormal specimens. Demonstrate the
        range by linear regression and 95% confidence
        intervals of the data points.

    c) Accuracy/Recovery Studies.
        Determine the spiking recovery of known amounts of
        the target lymphocyte subset (CDx). This may be
        accomplished by doing limiting dilutions of
        lymphocytes containing the subset of interest.

2. Reproducibility Studies.

    a) Intralaboratory Reproducibility.
        Determine the within-day precision using one flow
        cytometer, the new antibody and patient samples with
        three levels of CDx positive lymphocytes; high,
        normal, and low. Samples outside of the normal range
        can be obtained by diluting normal samples with known
        CDx negative cells. Run the samples in replicates of
        ten.

    b) Interlaboratory Reproducibility.
        Repeat the intralaboratory study at three independent
        sites.

        A summary of the results from the intra- and
        interlaboratory reproducibility studies should be
        reported in the Performance Characteristics Section of
        the Package Insert.

B. Clinical Comparison Studies.
Comparison studies provide data on the ability of the system
to accurately determine MAb immunophenotyping results as
compared to another legally marketed device. For comparison
of flow cytometry data, the clinical sites should furnish the
protocol for instrument calibration, instrument setup for
analysis, and isolation of cells of interest. Each site
performing the following studies should use the NCCLS
Standard, Clinical Applications of Flow Cytometry: Quality
Assurance and Immunophenotyping of Peripheral Blood
Lymphocytes.[1]

1. Specimen Collection and Handling.
Collect patient blood samples following the venipuncture
procedure outlined in the NCCLS Standard, Procedures for
the Collection of Diagnostic Blood Specimens by
Venipuncture.[4] For immunophenotyping, the
anticoagulants EDTA, ACD solution A, or heparin may be
used. Identify the blood specimen with a unique patient
identifier, date, time of collection, sex, age, and
pertinent medication information. Maintain blood samples
at 18-22°C and process within 6 hours of collection. For
flow cytometric analysis, samples may be fixed in a
solution of 1% paraformaldehyde or 1% buffered
formaldehyde, pH 7.2. State specimen storage conditions
in the Package Insert and provide data or appropriate
literature references in the submission to substantiate
any claims made.

2. Expected Normal Ranges.
Analyze anticoagulated whole blood samples from a minimum
of 50 normal donors from each of three geographically
diverse sites which may include the manufacturer's own
site. These donors should include all ethnic groups
(White, Asian, Hispanic, and Black, both U.S. and'
Caribbean) as well as represent both genders and a broad
defined age range. Test the blood samples with both the
new MAb and the comparable legally marketed MAb.

Test each cell isolation protocol claimed in the
Intended Use Statement with the 50 samples; e.g.,
Ficoll-hypaque mononuclear cell separation, buffy coat,
and whole blood lysis.

Establish a normal range of CDx positive lymphocytes
using the new test with samples from a minimum of 50
normal persons. Characterize the population study
according to age, sex, geographic location, and symptoms
of disease. State the statistical method used in the
package insert. All sample type(s)/matrices should be
considered in the studies.

3. **Abnormal Ranges.**
Support any claims or references to disease states with
data. Depending on the rarity of the disease, test a
minimum of 10 (more would be preferred) samples according
to the protocols submitted. The sampling for these
disease states should be as diverse as possible.
Continuous cell lines of the appropriate lineage may be
used to enhance the data if abnormal samples are
extremely hard to obtain. Determine the range of CDx
positive lymphocytes from each specific claimed disease
state.

4. **Flow Cytometer.**
If the intended use claim is not for a specific
manufacturer's flow cytometer, a comparison must be done
using both a Coulter and Becton-Dickinson instrument as
these are considered to be the most commonly used
instruments. For the comparison study use 20 to 30
patient samples which contain an evenly distributed
number of high, normal, and low levels of cells. Samples
outside of the normal range can be obtained by diluting
normal samples with known CDx negative cells. The
comparison samples should be the same for all instruments
and may consist of continuous cell lines possessing the
antigen of interest. Submit a representative number of
fluorescence intensity histograms for all flow cytometers
used.

C. **Presentation of Data.**
Present all data as percentage of the total lymphocyte
population, listed in tabular form and sorted based on the
submitted device.

Provide the linear regression for the comparison data with
the predicate device and, also, for each machine comparison.
List the mean channel fluorescence of the histograms for the
generic flow cytometer comparison data.

Include the calculations from the normal and disease states
for the mean, SD and the percent coefficient of variation
(CV).

Show the data from the disease states as a total and listed
by age, ethnic group, and sex.

Submit a representative number of fluorescence intensity
histograms for all flow cytometers used.

IV. Labeling Considerations.

A. **Suggested Intended Use Statement.**
A typical intended use statement is: "ABC's Bright Star
CDx murine monoclonal antibody is for the identification and
enumeration of CDx positive lymphocytes in whole blood using
flow cytometry."

B. **Clinical Utility.**
The clinical utility for the MAb should be included in the
Intended Use Statement.

C. **Limitation.**
The following limitation statement should be included in the
Package Insert:
Due to an unacceptable variance among the different
laboratory methods for determining absolute lymphocyte
counts, an assessment of the accuracy of the method used is
necessary.[5]

V. References.

1. National Committee for Clinical Laboratory Standards.
Clinical Applications of Flow Cytometry: Quality Assurance
and Immunophenotyping of Peripheral Blood Lymphocytes;
proposed guideline. Villanova, PA. 1989;9(13). Order code
H42-P.

2. Redfield RR, Wright DC, Tramont EC. The Walter Reed Staging
Classification for HTLV-III / LAV Infection. N Engl J Med
1986;314(2):131-132.

3. CDC. Classification System for Human T-Lymphotropic Virus
Type III / Lymphadenopathy - Associated Virus Infections.
MMWR 1986;35(2):334-339.

4. National Committee for Clinical Laboratory Standards.
Procedures for the Collection of Diagnostic Blood Specimens
by Venipuncture; approved standard. Villanova, PA.
1984;4(5). Order code H3-A2.

5. Koepke JA, Landay AL. Precision and Accuracy of Absolute
Lymphocytes Counts. Clin Immunology and Immunopathology
1989;52:19-27.