Complete Summary

GUIDELINE TITLE

Guidelines for performing single-platform absolute CD4+ T-Cell determinations with CD45 Gating for persons infected with human immunodeficiency virus.

BIBLIOGRAPHIC SOURCE(S)

Mandy FF, Nicholson JK, McDougal JS. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003 Jan 31;52(RR-2):1-13. [68 references] PubMed

GUIDELINE STATUS

This is the current release of the guideline.

COMPLETE SUMMARY CONTENT

SCOPE
METHODOLOGY - including Rating Scheme and Cost Analysis
RECOMMENDATIONS
EVIDENCE SUPPORTING THE RECOMMENDATIONS
BENEFITS/HARMS OF IMPLEMENTING THE GUIDELINE RECOMMENDATIONS
QUALIFYING STATEMENTS
IMPLEMENTATION OF THE GUIDELINE
INSTITUTE OF MEDICINE (IOM) NATIONAL HEALTHCARE QUALITY REPORT CATEGORIES
IDENTIFYING INFORMATION AND AVAILABILITY
DISCLAIMER

SCOPE

DISEASE/CONDITION(S)

Human immunodeficiency virus (HIV)

GUIDELINE CATEGORY

Diagnosis
Management

CLINICAL SPECIALTY

Pathology

INTENDED USERS

Clinical Laboratory Personnel

GUIDELINE OBJECTIVE(S)

- To supplement previous recommendations published in 1997 (1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus [HIV]. *MMWR* 1997;46[No. RR-2]) that describe dual-platform technology, a method in which absolute counts are derived from measurements obtained from two instruments—a flow cytometer and hematology analyzer
- To address concerns specific to the implementation of single-platform technology for CD4+ T-cell determination in persons infected with human immunodeficiency virus, as well as to address other general topics, such as laboratory safety and specimen handling

TARGET POPULATION

Persons infected with human immunodeficiency virus

INTERVENTIONS AND PRACTICES CONSIDERED

Single-platform technology absolute CD4+ T-cell determinations with CD45 gating, using three- or four-color monoclonal antibody panels

MAJOR OUTCOMES CONSIDERED

- Effectiveness of single-platform technology (SPT) to provide accurate and reliable measures of CD4+T lymphocytes in the laboratory
- Variability in laboratories' testing practices/procedures

METHODOLOGY

METHODS USED TO COLLECT/SELECT EVIDENCE

Searches of Electronic Databases

DESCRIPTION OF METHODS USED TO COLLECT/SELECT THE EVIDENCE

Not stated

NUMBER OF SOURCE DOCUMENTS

Not stated

METHODS USED TO ASSESS THE QUALITY AND STRENGTH OF THE EVIDENCE

Not stated

RATING SCHEME FOR THE STRENGTH OF THE EVIDENCE

Not applicable

METHODS USED TO ANALYZE THE EVIDENCE

Review

DESCRIPTION OF THE METHODS USED TO ANALYZE THE EVIDENCE

Not stated

METHODS USED TO FORMULATE THE RECOMMENDATIONS

Expert Consensus

DESCRIPTION OF METHODS USED TO FORMULATE THE RECOMMENDATIONS

These guidelines reflect a consensus of the third national conference on CD4+ immunophenotyping held on November 14-15, 2001 in Orlando, Florida. The conference was attended by representatives from public health, private, and academic laboratories as well as product manufacturers.

RATING SCHEME FOR THE STRENGTH OF THE RECOMMENDATIONS

Not applicable

COST ANALYSIS

A formal cost analysis was not performed and published cost analyses were not reviewed.

METHOD OF GUIDELINE VALIDATION

Not stated

DESCRIPTION OF METHOD OF GUIDELINE VALIDATION

Not stated

RECOMMENDATIONS

MAJOR RECOMMENDATIONS

I. **Laboratory Safety**
   A. Use universal precautions with all specimens (CDC, 1988).
   B. Adhere to the following safety practices (CDC, 1988; CDC, 2001; CDC, 1999):

1. Wear laboratory coats and gloves when processing and analyzing specimens, including reading specimens on the flow cytometer.
2. Never pipette by mouth. Use safety pipetting devices.
3. Never recap needles. Dispose of needles and syringes in puncture-proof containers designed for this purpose.
4. Handle and manipulate specimens (e.g., aliquot, add reagents, vortex, and aspirate) in a class I or II biological safety cabinet.
5. Centrifuge specimens in safety carriers.
6. After working with specimens, remove gloves and wash hands with soap and water.
7. For stream-in-air flow cytometers, follow the manufacturer's recommended procedures to eliminate the operator's exposure to any aerosols or droplets of sample material.
8. Disinfect flow cytometer wastes. Before adding waste materials to the waste container, add a sufficient volume of undiluted household bleach (5% sodium hypochlorite) so that the final concentration of bleach will be 10% (0.5% sodium hypochlorite) when the container is full (e.g., add 100 mL of undiluted bleach to an empty 1,000 mL container).
9. Disinfect the flow cytometer as recommended by the manufacturer. One method is to flush the flow cytometer fluidic chambers with a 10% bleach solution for 5--10 minutes at the end of the day and then flush with water or saline for at least 10 minutes to remove excess bleach, which is corrosive.
10. Disinfect spills with household bleach or an appropriate dilution of mycobactericidal disinfectant. **Note**: Organic matter will reduce the ability of bleach to disinfect infectious agents. National Committee for Clinical Laboratory Standards (NCCLS) recommendations regarding how to disinfect specific areas should be followed (National Committee for Clinical Laboratory Standards, 1991). For use on smooth, hard surfaces, a 1% solution of bleach is usually adequate for disinfection; for porous surfaces, a 10% solution is needed (National Committee for Clinical Laboratory Standards, 1991).
11. Ensure that all samples have been properly fixed after staining and lysing but before analysis. **Note**: Some commercial reagents employ a single-step, lyse and fix method that reduces the infectious activity of cell-associated human immunodeficiency virus (HIV) by 3--5 logs(Nicholson et al, 1993; Aloisio & Nicholson, 1990); however, these reagents have not been evaluated for their effectiveness against other agents (e.g., hepatitis virus). Cell-free HIV can be inactivated with 1% paraformaldehyde within 30 minutes (Cory, Rapp, & Ohlsson-Wilhelm, 1990; Lifson, Sasaki, & Engleman, 1986; Martin, Loskoski, & McDougal, 1987).

II. Specimen Collection for Single-Platform Technology

A. Anticoagulant

1. Use tripotassium ethylenediamine tetraacetate (K_3EDTA, 1.5 ± 0.15 mg/mL blood) or heparin (National Committee for Clinical Laboratory Standards, 1989; National Committee for Clinical Laboratory Standards, 1992; Nicholson & Green, 1993), and perform the test within the time frame allowed by the single-

platform technology (SPT) manufacturer. Because acid citrate dextrose is added as a liquid to blood collection tubes, its use would make calculating accurate final sample volume difficult and is not recommended. With this absolute counting technology, use of an accurate sample volume is critical.

2. Reject specimens that cannot be processed within 72 hours.

B. Collect blood specimens by venipuncture (National Committee for Clinical Laboratory Standards, 1998) into evacuated tubes containing K_3EDTA anticoagulant, completely expending the vacuum in the tubes.

1. Use pediatric tubes to obtain specimens from children, and ensure that the tube is full.
2. Mix the blood well with the anticoagulant to prevent clotting.

C. Label all specimens with the date, time of collection, and a unique patient identifier. Ensure that patient information and test results are accorded confidentiality.

III. **Specimen Transport**

A. Maintain and transport specimens at room temperature (64Â°--72Â°F [18Â°--22Â°C]) (Paxton & Bendele, 1993; Shield et al., 1983; McCoy, Carey, & Krause, 1990; Ekong et al., 1993). Specimens should not be exposed to extreme temperatures that could allow them to freeze or become too hot. Temperatures >99Â°F (37Â°C) might cause cellular destruction and affect flow cytometry measurements (Paxton & Bendele, 1993). In hot weather, pack the specimen in an insulated container. If necessary, place this container inside another containing an ice pack and absorbent material. This method helps retain the specimen at ambient temperature. The effect of cool temperatures (i.e., <39Â°F [4Â°C]) on CD45 gate-based immunophenotyping results is not clear (Paxton & Benedele, 1993; Ekong et al., 1993).

B. Transport specimens to the immunophenotyping laboratory as soon as possible.

C. For transport to locations outside the collection facility, follow state or local guidelines. One method for packaging such specimens is to place the tube containing the specimen in a leakproof container (e.g., a sealed plastic bag) and to pack this container inside a cardboard canister containing sufficient material to absorb all the contents should the tube break or leak. Cap the canister tightly. Fasten the request slip securely to the outside of this canister with a rubber band. For mailing, this canister should be placed inside another canister containing the mailing label.

D. For interstate shipment, follow federal guidelines for transporting diagnostic specimens (available at http://www.cdc.gov/od/ohs/biosfty/shipregs.htm). **Note**: Use overnight carriers with an established record of consistent overnight delivery to ensure arrival the following day. Check with these carriers for their specific packaging requirements.

E. Obtain specific protocols and arrange appropriate times of collection and transport from the facility collecting the specimen.

IV. **Specimen Integrity**

A. Inspect the tube and its contents immediately upon arrival.

B. Take corrective actions if any of the following occur:

1. If the specimen is hot or cold to the touch but not obviously hemolyzed or frozen, process it but note the temperature condition on the worksheet and report form. Do not rapidly

warm or chill specimens to bring them to room temperature because this may adversely affect the immunophenotyping results (Paxton & Bendele, 1993). Abnormalities in light-scattering patterns may reveal a compromised specimen.

2. If blood is hemolyzed or frozen, reject the specimen and request another.
3. If clots are visible, reject the specimen and request another.
4. If the specimen is received >72 hours after collection, reject it and request another.

V. **Specimen Processing**

A. Perform the test within 48 hours (preferred), but no later than 72 hours after drawing the blood specimen (Bergeron et al., 2002).
B. Place the samples on a gentle blood rocker for 5 minutes to ensure that the samples are uniformly distributed.
C. Pipette blood volumes accurately and in a reproducible manner. A reverse pipetting technique is recommended (Refer to information box in original guideline document).
D. Vortex sample tubes to mix the blood and reagents and break up cell aggregates. In addition, vortex samples immediately after the lyse/fixation step and before analysis to disperse cells optimally.
E. Incubate all tubes in the dark during the staining procedure.
F. A lyse/no-wash method is required for SPT. Follow directions provided by the manufacturer.
G. Immediately after processing the specimens, cap the tubes and store all stained samples in the dark and under refrigeration (39Â°–50Â°F [4Â°–10Â°C]) until flow cytometric analysis. These specimens should not be stored for longer than 24 hours unless the laboratory can demonstrate that scatter and fluorescence patterns do not change for specimens for stored longer periods.

VI. **Monoclonal Antibody Panels**

A. CD45 is required to aid in the identification of lymphocytes. Lymphocytes are brightly positive for CD45 and have low light-scattering characteristics.
B. Monoclonal antibody panels must contain appropriate antibody combinations to enumerate CD4+ and CD8+ T-cells and to ensure the quality of the results (Refer to Table 1 in original guideline document).
   1. CD4 T-cells are identified as being positive for CD3 and CD4.
   2. CD8 T-cells are identified as being positive for CD3 and CD8.
C. Three-color monoclonal antibody panels
   1. Three-color monoclonal antibody panels should fulfill the following basic requirements: enumerate CD4+ and CD8+ T-cells, validate the CD45 gate used, and provide some assessment of tube-to-tube variability.
   2. Three-color monoclonal antibody panels must consist of at least two tubes, each with the same lineage marker. For the examples described previously, CD3 is the common lineage marker in each tube. Differences between replicate CD3 results should be $\leq$2%.
   3. CD19+ B-cell values may be important in assessing immune status of pediatric patients.
D. Four-color monoclonal antibody panels
   1. Addition of CD45 to a single tube containing CD3, CD4, and CD8 allows the identification of lymphocytes based on CD45

and side scatter and the enumeration of CD4+ and CD8+ T-lymphocytes.

2. CD19+ B-cell values may be essential for assessing the immune status of pediatric patients.
3. Use of a second tube containing a natural killer (NK) cell marker together with CD3 and CD19 can help to assess the recovery and purity of the lymphocytes within the CD45/side-scatter gate.

VII. Negative and Positive Controls for Immunophenotyping

Note: An isotype control is not needed.

A. Positive methodologic control
   1. Use the methodologic control to determine whether procedures for preparing and processing the specimens are optimal. Prepare this control each time specimens from patients are prepared.
   2. Use either a whole-blood specimen from a control donor or commercial materials validated for this purpose.
   3. If the methodologic control falls outside established normal ranges, determine the reason. **Note**: The purpose of the methodologic control is to detect problems in preparing and processing the specimens. Biologic factors that cause only the whole-blood methodologic control to fall outside normal ranges do not invalidate the results from other specimens processed at the same time. Poor lysis or poor labeling in all specimens, including the methodologic control, invalidates results.

B. Positive control for evaluating reagents
   1. Use the positive control to test the labeling efficiency of new lots of reagents or when the labeling efficiency of the current lot is questioned. Prepare this control only when needed (i.e., when reagents are in question) in parallel with reagent lots of known acceptable performance. **Note**: New reagents must demonstrate similar results to those of known acceptable performance.
   2. Use a whole-blood specimen or other human lymphocyte preparation (e.g., cryopreserved or commercially obtained lyophilized lymphocytes or stabilized whole blood).

VIII. Flow Cytometer Quality Control

A. Verify optical alignment daily. Usually, clinical flow cytometers that are capable of three- and four-color immunophenotyping have fixed optical systems, i.e., the relative position of the flow cell with respect to the optical elements is fixed. In such systems, the instrument operator cannot optimize alignment but must verify that the instrument meets the manufacturer's specifications for optical alignment. Regardless of whether the alignment is user adjustable, it should be checked with alignment standards, such as wide-spectrum fluorescent microfluorospheres with measurable light-scatter characteristics. Daily monitoring of optical alignment ensures that the cytometer gives acceptably bright fluorescence measurements and that homogeneous peaks are produced for all parameters to be used in sample analysis (National Committee for Clinical Laboratory Standards, 1998).

1. Use a stable calibration material (e.g., microfluorospheres labeled with fluorochromes) that has measurable and known forward-scatter, side-scatter, and fluorescence properties in each channel to be used for sample analysis.
2. Verify acceptable optical alignment by establishing that calibration particles meet manufacturer- or laboratory-defined criteria for brightness and homogeneity.
3. Align stream-in-air flow cytometers daily (at a minimum) and stream-in-cuvette flow cytometers (most clinical flow cytometers are this type) as recommended by the manufacturer.

B. Standardize fluorescence and light-scatter signals daily. This ensures that the flow cytometer is operating within manufacturer- or laboratory-defined acceptance ranges under test-specific conditions each day and that its performance is consistent from day to day.
1. Select machine settings that are appropriate for antibody/fluorochrome-labeled, whole-blood specimens.
2. Use microfluorospheres or other stable standardization material to place the scatter and fluorescence peaks in the same narrow range of scatter and fluorescence channels each day. Adjust the flow cytometer as needed.
3. Retain machine standardization settings for the remaining quality control procedures (sensitivity and color compensation) and for reading the specimens.

C. Determine fluorescence resolution daily. The flow cytometer must differentiate between the dim peak and autofluorescence in each fluorescence channel (National Committee for Clinical Laboratory Standards, 1998).
1. Unstained and lysed fresh whole blood is suitable for adjusting the photomultiplier tube (PMT) voltages. The autofluorescence from the unstained lymphocytes should be completely on scale (i.e., <5% of cells within the lymphocyte light-scatter gate fall in channel 0 in each fluorescence scale) and should fall within the lower left quadrant of the dot plot for every PMT/detector in use.
2. Evaluate standardization/calibration material or cells to verify that cells with low-level fluorescence can be resolved from autofluorescence (e.g., microbeads with low-level and negative fluorescence, CD56-labeled lymphocytes, or dim cells in CD8-labeled lymphocytes).
3. Establish a minimal acceptable distance between peaks; monitor this difference, and correct any daily deviations.

D. Compensate for spectral overlap daily (See Figure 1 in the original guideline document). Compensation is the process of correcting for spectral overlap of one fluorochrome into the filter window being used to monitor another fluorochrome. In most instruments used clinically, this correction is done by adjusting the electronic compensation circuits on the flow cytometer to place populations not expected to be dual positive for two fluorochromes into orthogonal fluorescence quadrants with no overlap into the double-positive quadrant. At the same time, avoiding overcompensation is essential because this may cause dual-positive cells to be incorrectly classified as single positive. The following procedures may be performed manually, or the software

on the flow cytometer may perform the spectral compensation automatically.

1. Select the compensation control so it will match the brightest specimen signal. Use either microbead or cellular compensation material containing four populations for three-color immunofluorescence (no fluorescence, phycoerithrin [PE] fluorescence only, fluorescein isothiocyanate [FITC] fluorescence only, and a population that is positive for only the third color) or five populations for four-color (the four described previously and a population that is positive for only the fourth color).
2. Analyze this material, and adjust the electronic compensation circuits on the flow cytometer to place the fluorescent populations in their respective fluorescence quadrants with no overlap into the double-positive quadrant (Refer to Figure 1 in original guideline document). With three fluorochromes, compensation must be carried out in an appropriate sequence: FITC, PE, and the third color, respectively (Mandy et al., 1992). For four-color monoclonal antibody panels, follow the flow cytometer manufacturer's instructions for four fluorochromes. Avoid overcompensation.
3. If standardization or calibration particles (microbeads) have been used to set compensation, confirm proper calibration by using lymphocytes labeled with FITC- and PE-labeled monoclonal antibodies and a third-color- or fourth-color-labeled monoclonal antibody for three-color or four-color panels, respectively (Mandy et al., 1992). So that separate cell populations can be recognized without overlap, cells in individual tubes may be separately stained with each different fluorochrome-labeled antibody and then combined in a single tube for analysis. These populations should have the brightest expected signals. **Note**: Using a dimmer-than-expected signal to set compensation can result in suboptimal compensation for the brightest signal.
4. Reset compensation when photomultiplier tube voltages or optical filters are changed.
5. Commercially available software can analyze data without compensation and perform the compensation automatically. When using this software, follow manufacturer's instructions for this procedure.

E. Repeat all four instrument quality control procedures (section VIII A–C) whenever instrument problems occur or if the instrument has been serviced.

F. Maintain instrument quality control logbooks and monitor them continually for changes in any of the parameters. In the logbook, record instrument settings, peak channels, and coefficient of variation (CV) values for materials used to monitor or verify optical alignment, standardization, fluorescence resolution, and spectral compensation. Reestablish target fluorescence levels for each quality control procedure when lot numbers of beads are changed or the instrument has been serviced.

IX. Sample Analyses

A. With single-platform absolute count determination, use of the lyse/no-wash sample processing is mandatory. The lymphocyte population is identified as having bright CD45 fluorescence and low side-scattering properties (See Figure 2 in the original guideline document). Set the threshold or discriminator as recommended by the manufacturer. Adjust side scatter so that all leukocyte populations are visible. Draw a gate on the bright CD45+ cell population and analyze the cells in that population (Nicholson, Hubbard, & Jones, 1996).

B. Count at least 2,500 gated lymphocytes in each sample to ensure that enough cells and beads have been counted to provide an accurate absolute lymphocyte value.

X. **Data Analysis**

A. CD45 gating

1. Lymphocytes are identified by being brightly labeled with CD45 monoclonal antibody and having low side-scattering properties. Two typical examples of a four-color SPT analysis based on CD45 gating are illustrated (See Figure 2 in the original guideline document).
2. Establish criteria for cluster identification based on a clear definition of lymphocytes that does not include basophils (less bright CD45, low side scatter) or monocytes (less bright CD45, moderate side scatter). **Note**: Care must be taken to include all lymphocytes. CD45 fluorescence may be slightly less with B cells than with T cells (the major cluster of lymphocytes). NK cells have bright CD45 fluorescence but have slightly more side-scattering properties than the majority of the lymphocytes.
3. CD45/side-scatter gates for lymphocytes are assumed to contain >95% lymphocytes. Lymphocyte purity is assumed to be high with the CD45/side-scatter gating strategy; therefore, correction of lymphocyte subset values is not needed (Nicholson, Hubbard, & Jones, 1996).
4. If an estimate of lymphocyte recovery is needed (i.e., percentage of total lymphocytes within the CD45/side scatter gate), all the B and NK cells must be immunophenotyped as well. **Note**: Validation of a CD45/side-scatter gate is recommended during its initial use to help determine the CD45 and side-scatter characteristics of T, B, and NK cells and to ensure their inclusion in the gate.

B. Set cursors based on the tube containing CD3/CD4 and CD3/CD8 so that the negative and positive cells in the histogram are clearly separated.

C. Analyze each patient or control specimen with lymphocyte gates and cursors for positivity set for that particular patient or control.

D. Include the following analytic reliability checks, when available:

1. With single-platform technology (SPT), an additional analytical tool can be used to check the accuracy of the absolute count; time can be used as a parameter to determine how long it takes to obtain a microfluorosphere count that represents a unit volume of blood analyzed. Optimally, if blood pipetting was performed without noticeable error and the beads were accurately added to the tubes, the time required to analyze a microliter of whole blood should be constant. Follow manufacturer's instructions to set time as an active parameter.

If more or less time is required for a sample to accumulate the usual number of microspheres, this may indicate a serious counting problem and specimen processing should be repeated.

2. Optimally, the sum of the percentages of CD3+CD4+ and CD3+CD8+ cells should equal the total percentage of CD3+ cells ± 5%, with a maximum variability of <10%. Note: For specimens containing a considerable number of T gamma delta T-cells (Margolick et al., 1991; DePaoli, 1991), this reliability check may exceed the maximum variability.

XI. **Data Storage**

A. Store list-mode data for all specimens analyzed. This allows for reanalysis of the raw data, including redrawing of gates. At a minimum, retain hard copies of the CD45/side-scatter gate and correlated dual-histogram data of each sample's fluorescence.

B. Retain all primary files, worksheets, and report forms for 2 years or as required by state or local regulation, whichever is longer. Data can be stored electronically. Disposal after the retention period is at the discretion of the laboratory director.

XII. **Data Reporting**

A. Report all data in terms of CD designation, with a short description of what that designation means. **Note**: CD4+ T cells are T-helper cells. The correct cells to report for this value are those that are positive for both CD3 and CD4. Similarly, CD8+ T-cells are T-suppressor/cytotoxic cells and are positive for both CD3 and CD8. Do not include other cell types (non-T cells) in CD4 and CD8 T-cell determinations.

B. Report lymphocyte subset values as follows:

1. Report both percentages and absolute counts.
2. With SPT, determine the absolute counts directly from the flow cytometers. These calculations are usually handled by software that reports calculated results. The following formula should be used:

$$\frac{\text{No. of events in the bright CD45 region}}{\text{No. of events in the microfluorosphere region}}$$

$$\frac{\text{Total no. of microfluorospheres added}}{\text{Volume of blood added}}$$

C. Report data from all relevant monoclonal antibody combinations with corresponding reference limits of expected normal values (e.g., CD4+ T-cell absolute number and percentage). Reference limits for immunophenotyping test results must be determined for each laboratory (National Committee for Clinical Laboratory Standards, 1998). Separate reference ranges must be established for adults and children, and the appropriate ranges must be reported for patient specimens.

XIII. **Quality Assurance**

A. Ensure the overall quality of the laboratory's CD4+ T-cell testing by monitoring and evaluating the effectiveness of the laboratory policies and procedures for the preanalytic, analytic, and postanalytic testing phases. The practices and processes to be monitored and evaluated include the following:

- methods for collecting, handling, transporting, identifying, processing, and storing specimens
- information provided on report forms for test requests and results
- instrument performance, quality control protocols, and maintenance
- reagent quality control protocols
- process for reviewing and reporting results
- employee training and education, which should consist of the following:
    - basic training by flow cytometer manufacturers and additional training involving hands-on workshops for flow cytometer operators and supervisors
    - education of laboratory directors regarding flow cytometric immunophenotyping through workshops and other programs
    - continuing education regarding new developments for all flow cytometric immunophenotyping personnel through meetings and workshops
    - adherence to federal and state regulations for training and education
- assurance of satisfactory performance. Laboratories must fully participate in a performance evaluation program and demonstrate acceptable level of performance. When proficiency testing programs have been approved by the Centers for Medicare & Medicaid Services (formerly, the Health Care Financing Administration) as meeting the requirements of the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) (none are currently approved for CD4+ T-cell testing), laboratories must satisfactorily participate.
- review and revision (as necessary or at established intervals) of the laboratory's policies and procedures to ensure adherence to the quality assurance program. All staff involved in the testing should be informed of any problems identified during the quality assurance review, and corrective actions should be taken to prevent recurrences.

B. Document all quality assurance activities.

CLINICAL ALGORITHM(S)

Not applicable

EVIDENCE SUPPORTING THE RECOMMENDATIONS

REFERENCES SUPPORTING THE RECOMMENDATIONS

References open in a new window

TYPE OF EVIDENCE SUPPORTING THE RECOMMENDATIONS

The evidence supporting the recommendations is stated throughout the body of the guideline.

BENEFITS/HARMS OF IMPLEMENTING THE GUIDELINE RECOMMENDATIONS

POTENTIAL BENEFITS

The use of guideline recommendations for single-platform technology (SPT) might foster improved laboratory practices. Outcomes associated with SPT and CD45 gating include a) increased confidence in results, b) more reproducible results, c) increased ability to resolve discrepant problems, d) decreased proportion of unacceptable specimens received for testing, e) decreased proportion of specimens requiring reanalysis, and f) fewer incidents that could pose biohazard risks.

POTENTIAL HARMS

Not stated

QUALIFYING STATEMENTS

QUALIFYING STATEMENTS

- Although these guidelines for single-platform technology (SPT) use might foster improved laboratory practices, developing comprehensive guidelines for every aspect of CD4+ T-cell testing (including some laboratory-specific practices) is not possible. Moreover, measuring the outcomes associated with the adoption of these guidelines is inherently difficult. First, the guidelines lack evaluation protocols that can adequately account for the interactions among the recommendations. No weight of importance has been assigned for the individual recommendations that address unique steps in the testing process; hence, the consequences of incompletely following the entire set of recommendations are uncertain. Second, because published data are not available for every aspect of the guidelines, certain recommendations are based on the experience and opinion of knowledgeable persons. Recommendations made on this basis, in the absence of data, may be biased and inaccurate. Finally, variations in testing practices and interactions among the practices (e.g., how specimens are obtained and processed, skill of laboratory personnel [such as with pipetting], testing methods used, test-result reporting practices, and compliance with other voluntary standards and laboratory regulations) complicate both the development of guidelines that will fit every laboratory's unique circumstances and the assessment of the value of implementing the guidelines.
- These guidelines for SPT are intended for domestic implementation. Several alternative methods are available that require fewer reagents and involve more cost-effective gating algorithms. Some of these alternative methods may be compatible with current U.S. clinical laboratory methods; however, to date they have not been validated for domestic applications. As published validation data accumulate from multisite studies for methods such as Pan*Leuco*gating and primary CD4 gating, these potentially more cost-effective options will be considered as alternative or substitute methods. In the future,

guidelines should be harmonized to include all methods that meet domestic performance standards to ensure consistent high quality.

IMPLEMENTATION OF THE GUIDELINE

DESCRIPTION OF IMPLEMENTATION STRATEGY

Evaluation and Validation of a Newly Adopted Single-Platform Technology in the Laboratory

When a laboratory adopts the new single-platform technology (SPT), specimens should be tested in parallel by using both the current and the new method to characterize any systematic differences in the methods. Laboratorians should use statistical tools that provide useful information for the comparison studies. Linear least squares regression analyses are helpful in establishing good correlations between the new and established methods. If no error is detected with the new method, the r^2 value will approach 1.0. However, regression-type scatter plots provide inadequate resolution when the errors are small in comparison with the analytical range and do not characterize the relationship between the two methods.

A bias scatter plot provides laboratorians with a more useful tool for determining bias. These simple, high-resolution graphs plot the differences in the individual measurements of each method (result of old method--result of new method) against measurements obtained with one of the methods (result of old method). Such graphs provide an easy means of determining if bias is present and distinguishing whether bias is systematic, proportional, or random/nonconstant. The laboratorian can visually determine the magnitude of these differences over the entire range of values. When sufficient values are plotted, outliers or samples containing interfering substances can be identified. The laboratorian can then divide the data into ranges relevant to medical decisions and calculate the systematic error (mean of the bias) and the random error (standard deviation of the bias) to gain insight into analytical performance at the specified decision points.

Several detailed guidelines and texts provide additional information regarding quality goals, method evaluation, estimation of bias, and bias scatter plots (refer to the original guideline document for relevant citations). Once a new method is accepted and implemented, the laboratory will need to confirm or redefine its normal range and should continue to monitor the correlation between the results and the patient's clinical disease data to ensure that no problems have gone undetected by the relatively few samples typically tested during method evaluations.

INSTITUTE OF MEDICINE (IOM) NATIONAL HEALTHCARE QUALITY REPORT CATEGORIES

IOM CARE NEED

Living with Illness
Staying Healthy

IOM DOMAIN

Effectiveness

IDENTIFYING INFORMATION AND AVAILABILITY

BIBLIOGRAPHIC SOURCE(S)

Mandy FF, Nicholson JK, McDougal JS. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003 Jan 31;52(RR-2):1-13. [68 references] PubMed

ADAPTATION

Not applicable: The guideline was not adapted from another source.

DATE RELEASED

2003 Jan 31

GUIDELINE DEVELOPER(S)

Centers for Disease Control and Prevention - Federal Government Agency [U.S.]

SOURCE(S) OF FUNDING

United States Government

GUIDELINE COMMITTEE

Not stated

COMPOSITION OF GROUP THAT AUTHORED THE GUIDELINE

Prepared by: Francis F. Mandy, PhD (Bureau of HIV/AIDS, STD and TB Population and Public Health Branch, Health Canada, Ottawa, Ontario, Canada); Janet K.A. Nicholson, PhD (Office of the Director); J. Steven McDougal, MD (Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, CDC)

FINANCIAL DISCLOSURES/CONFLICTS OF INTEREST

Not stated

GUIDELINE STATUS

This is the current release of the guideline.

GUIDELINE AVAILABILITY

Electronic copies: Available from the Centers for Disease Control and Prevention (CDC) Web site:

- HTML Format
- Portable Document Format (PDF)

Print copies: Available from the Centers for Disease Control and Prevention, MMWR, Atlanta, GA 30333. Additional copies can be purchased from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402-9325; (202) 783-3238.

AVAILABILITY OF COMPANION DOCUMENTS

None available

PATIENT RESOURCES

None available

NGC STATUS

This NGC summary was completed by ECRI on February 12, 2003.

COPYRIGHT STATEMENT

No copyright restrictions apply.

DISCLAIMER

NGC DISCLAIMER

The National Guideline Clearinghouse™ (NGC) does not develop, produce, approve, or endorse the guidelines represented on this site.

All guidelines summarized by NGC and hosted on our site are produced under the auspices of medical specialty societies, relevant professional associations, public or private organizations, other government agencies, health care organizations or plans, and similar entities.

Guidelines represented on the NGC Web site are submitted by guideline developers, and are screened solely to determine that they meet the NGC Inclusion Criteria which may be found at http://www.guideline.gov/about/inclusion.aspx .

NGC, AHRQ, and its contractor ECRI Institute make no warranties concerning the content or clinical efficacy or effectiveness of the clinical practice guidelines and related materials represented on this site. Moreover, the views and opinions of developers or authors of guidelines represented on this site do not necessarily state or reflect those of NGC, AHRQ, or its contractor ECRI Institute, and inclusion

or hosting of guidelines in NGC may not be used for advertising or commercial endorsement purposes.

Readers with questions regarding guideline content are directed to contact the guideline developer.

© 1998-2008 National Guideline Clearinghouse

Date Modified: 9/22/2008