

**NHANES 1999–2000 Data Release  
December 2004  
Documentation for Laboratory Results**

**Laboratory 3 – HIV antibody test result, CD4+ T-lymphocytes (helper T cells), and  
CD8+ T cells (suppressor/inducer T cells)**

**(1) Documentation File UpDate – August 2006**

**(2) Documentation File Name – Laboratory 3 - HIV antibody test result, CD4+ T-lymphocytes (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

**(3) Survey Years Included in this File Release – 1999–2000**

**(4) Component Description**

**HIV antibody test result**

The estimated prevalence of human immunodeficiency virus (HIV) infection in the United States population is an important measure of the extent of the medical and financial burden the nation faces because of this virus. The current NHANES and HIV antibody data from NHANES III (1988-94) serve as a baseline for monitoring the changes in the epidemic over time in the general population of the United States. In addition to HIV antibody testing in NHANES, whole-blood samples were collected and stored for future CD4 testing once the HIV status of the sample was known. This procedure allows CDC to determine the distribution of CD4 cells in a random sample of HIV-positive individuals. NHANES is now the only national survey collecting blood on a population-based sample; therefore, it will be a key element in future estimates. If the participant refused phlebotomy, but did not refuse the HIV test, urine was tested for HIV antibody.

**CD4+ T-lymphocytes (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

HIV infection is characterized by a decrease and, eventually, a depletion of CD4+ T-lymphocytes (helper T cells). Using immunophenotyping, HIV-positive blood samples and age-matched controls were tested for the proportion of lymphocytes that are T cells, B cells, natural killer (NK) cells, CD4+ T cells (helper T cells), and CD8+ T cells (suppressor/inducer T cells).

**(5) Sample Description**

**HIV antibody test result**

All specimens were tested using the Synthetic Peptide Enzyme Immunoassay (EIA) (Genetic Systems HIV-1/HIV-2 Peptide EIA) for the detection of antibody to human immunodeficiency virus type 1 or type 2 (HIV-1 and HIV-2) or both (Bio-Rad Laboratories, Hercules, CA). Any specimen that reacted in an initial test was retested in duplicate with the Genetic Systems HIV-1/HIV-2 Peptide EIA. Initially reactive specimens that were reactive in either one or both duplicates from the repeat testing are referred to as “repeatedly reactive”. These repeatedly reactive specimens were then tested with a more specific test, the Cambridge Biotech HIV-1 Western Blot Kit (Calypse Biomedical Corporation, Rockville, MD).

## **CD4+ T-lymphocytes (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

Whole EDTA anti-coagulated blood stored at  $-70^{\circ}\text{C}$  from the 31 HIV antibody-positive participants from NHANES 1999-2004 and 34 age-matched controls were used.

Enumeration of CD4+ lymphocytes in HIV-positive participants and age-matched controls was performed on cryopreserved whole blood using the method reported by Fiebig et. al. (11). Samples were batch-thawed quickly in 8-10 samples per batch in a  $37^{\circ}\text{C}$  water bath and analyzed within 2 hours of set-up. The CD4 cell counts were obtained by using the Becton Dickinson MultiTEST reagent in TrueCOUNT tubes (Becton Dickinson Immunocytometry Systems, San Jose, CA). Comparison of this method with CD4 counts calculated from CBC absolute lymph counts from fresh whole blood X CD4% from the frozen whole blood resulted in a correlation coefficient of 0.9872.

### **5.1 Eligible Sample**

Participants aged 18–49 years who did not refuse the HIV antibody test and had a blood sample available for CD4+ testing were considered eligible for testing.

## **(6) Description of the Laboratory Methodology**

### **HIV antibody test result**

#### **1. Blood assay**

All specimens were tested using the Synthetic Peptide Enzyme Immunoassay (EIA) (Genetic Systems HIV-1/HIV-2 Peptide EIA) for the detection of antibody to human immunodeficiency virus type 1 or type 2 (HIV-1 or HIV-2) or both (Bio-Rad Laboratories, Redmond, WA). Any specimen that reacted in an initial test was retested in duplicate with the Genetic Systems HIV-1/HIV-2 Peptide EIA. Initially reactive specimens that were reactive in either one or both duplicates from the repeat testing are referred to as “repeatedly reactive”. These repeatedly reactive specimens were then tested with a more specific test, the Cambridge Biotech HIV-1 Western Blot Kit (Calypte Biomedical Corporation, Rockville, MD).

The combination of electrophoretic separation of complex mixtures of antigens with the highly sensitive immunoblotting technique has been useful in characterizing the antigenic profile of HIV-1 and describing the immune response to this virus in exposed or infected persons.

The Cambridge Biotech HIV-1 Western Blot Kit, when used as directed, will detect antibodies to HIV-1 when present in human serum or plasma. The position of bands on the nitrocellulose strips allows this antibody reactivity to be associated with specific viral antigens.

The Cambridge Biotech HIV-1 Western Blot Kit is manufactured by Calypte Corporation from HIV-1 propagated in an H9/HTLV-IIIb T-lymphocyte cell line. The partially purified virus is inactivated by treatment with psoralen, ultraviolet light, and detergent disruption. Specific HIV-1 proteins are fractionated according to molecular weight by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecyl sulfate (SDS).

The separated HIV-1 proteins are electrotransferred from gel to a nitrocellulose membrane, which is then washed, blocked (to minimize nonspecific immunoglobulin binding), and packaged. Individual nitrocellulose strips are incubated with serum or plasma specimens, or controls. During incubation, if HIV-1 antibodies are present in the specimen, they will bind to the viral antigens bound to the nitrocellulose strips. The strips are washed again to remove unbound material.

Visualization of the human immunoglobulins specifically bound to HIV-1 proteins is accomplished *in situ* by using a series of reactions with goat anti-human IgG conjugated with biotin, avidin conjugated with horseradish peroxidase (HRP), and the HRP substrate 4-chloro-1-naphthol. If antibodies to any of the major HIV-1 antigens are present in the specimen in sufficient concentration, bands corresponding to the position of one or more of the following HIV-1 proteins (p) or glycoproteins (gp) will be seen on the nitrocellulose strip: p17, p24, p31, gp41, p51, p66, gp120, gp160 (number refers to apparent molecular mass in kilodaltons).

## **2. Urine assay**

Eligible individuals who refused phlebotomy or who did not have a sufficient blood sample for the serum HIV assay but who did not refuse HIV testing had their urine tested for HIV type 1 antibody using the Calypte HIV-1 Urine EIA. As with serum, initially reactive samples were retested in duplicate, and all repeatedly reactive samples were confirmed with a Western blot. The Cambridge Biotech HIV-1 urine Western Blot kit (Calypte biomedical, Rockville, MD) was used to confirm positive tests. The specifics of the urine assay are similar to the blood Western blot (see above).

## **Whole-blood CD4+ T cells (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

Using immunophenotyping, the NHANES specimens were tested for the proportion of lymphocytes that are CD4+ T cells (helper T cells) and CD8+ T cells (suppressor/inducer T cells). This was done by incubating anti-coagulated whole blood with fluorescence-labeled monoclonal antibodies to the various cellular antigens that identify specific cell populations (phenotypes), then lysing the blood to remove red blood cells. The antibodies were conjugated to fluorescent tags that emit light of a certain frequency when excited by a laser beam. The specimens were analyzed on a flow cytometer to determine the proportion of lymphocytes of a particular phenotype (that emit light at the right wavelength).

The TruCount method (MultiTEST 4-color) uses TruCount tubes which contain a lyophilized pellet containing a known quantity of fluorescent beads. A precise quantity of whole blood is added to the tubes, and the lymphocytes are stained with MultiTEST monoclonal antibodies. The absolute count of a full lymphocyte subset profile (CD3+, CD3+CD4+, CD3+CD8+, CD3-CD19+, CD3-CD16/56+) can be determined in four tubes with Trieste and two tubes with MultiTEST by calculating the ratio of region events for each subset to bead events using the BD Biosciences-developed software, Mustiest. The one-tube panel (CD3+ CD4+ and CD3+ CD8+) was done on the NHANES samples.

## **(7) Laboratory Quality Control and Monitoring**

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the [NHANES Laboratory/Medical Technologists Procedures Manual \(LPM\)](#). Read the LABDOC file for detailed QA/QC protocols.

## **CD4+ T cells (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

1. A freshly drawn peripheral whole-blood specimen from a healthy donor was run as a daily quality control with each run of NHANES samples for RBC-lysing control.
2. CD-Chex Plus Normal control (Streck Labs #213326) was run using each antibody for precision of absolute counts.

Reagent login: when a new batch of reagents (whole-blood control, TruCOUNT controls, TruCOUNT tubes and antibodies, CaliBRITE beads, etc.) was received, it was logged in on the appropriate reagent record sheet recording the batch number, lot number, quantity, expiration date, date received, and initials. The batch number was written in red on the reagent vial. When a new batch was opened, the date of first use was recorded and an expected range of values ( $\pm$ ) was recorded on the appropriate QC sheet in the TruCOUNT QA/QC notebook; a copy of the package insert was placed in the TruCOUNT 3-Color and 4-Color QA/QC book. Prior to putting new lots of reagents or kits into use, a lot-to-lot reagent check was performed in parallel with the current lot in use. These results were recorded on the reagent login sheet. If results of the new lot were not within 5% of the previous lot, the run was repeated with different specimens and, if still out of this range, the lot was not used.

3. For frozen whole-blood control, a normal whole-blood sample with known values that had been stored in 0.5-ml aliquots at  $-70^{\circ}\text{C}$  and tested for CD3+ CD4+ and CD3+ CD8+ counts when freshly collected was used. An aliquot was removed from the freezer, thawed, and tested with each NHANES run.

## **(8) Data Processing and Editing**

### **HIV antibody test result**

Blood and urine specimens were processed, stored, and shipped to the Division of AIDS, STD, and TB, National Center for HIV, STD, and TB Prevention, National Centers for Disease Control and Prevention. Detailed specimen collection and processing instructions are discussed in the LPM. Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.

## **CD4+ T cells (helper T cells) and CD8+ T cells (suppressor/inducer T cells)**

Data was received after all the HIV testing was complete. The data on these results were not edited.

### **(9) Data Access:**

All data are publicly available.

### **(10) Analytic Notes for Data Users:**

The serum specimens were first tested by enzyme immunoassay (EIA) and confirmed by Western blot (WB). If the EIA was repeatedly negative, the HIV antibody result was coded as negative. If the EIA was positive and the WB was positive, the result was coded as positive. If the EIA was positive or indeterminate but the WB was negative, the result was coded as negative. If the EIA was positive or indeterminate but the WB was indeterminate, the result was coded as indeterminate.

CD4+ counts are only available on HIV-positive samples with available blood and age-matched controls.

## **(11) References**

1. Morbidity and Mortality Weekly Report (MMWR); Guidelines for the performance of CD4+ T cell determinations in persons with human immunodeficiency virus infection. May 8, 1992, Vo1.41, No.RR-8.
2. Morbidity and Mortality Weekly Report (MMWR). Guidelines for performing single-platform absolute CD4+ T cell determinations with CD45 gating in persons with human immunodeficiency virus. January 31, 2003, Vo1.52, No.RR-2.
3. National Committee for Clinical Laboratory Standards (NCCLS). Clinical applications of flow cytometry. Quality assurance and immunophenotyping of peripheral blood lymphocytes. NCCLS H42-T, 1992.
4. Becton Dickinson TriTest and MultiTEST Reagent package inserts.
5. Becton Dickinson Monoclonal Antibody Source Book.
6. National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS, Flow Cytometry Advisory Committee. Guidelines for flow cytometric immunophenotyping, Version 1.0, January 1993.
7. Nicholson, JKA, Jones, BM, Hubbard, M. CD4 T-lymphocyte determinations on whole-blood specimens using a single-tube three-color assay. *Cytometry*. 1993;14:685-689.
8. Nicholson, JKA. Immunophenotyping specimens from HIV-infected persons: laboratory guidelines from the Centers for Disease Control and Prevention. *Cytometry*. 1994;18:55-59.
9. Nicholson, JKA, Hubbard, M, Jones, BM. Use of CD45 fluorescence and side-scatter characteristics for gating lymphocytes when using the whole-blood lysis procedure and flow cytometry. *Cytometry*. 1996;26:16-21.
10. Nicholson, JKA, Hubbard, M, Dawson, C. Evaluation of stabilized whole-blood control materials for lymphocyte immunophenotyping. *Cytometry*. 1999;38:268-273.
11. Fiebig, EW, Johnson, DK, Hirschorn, DF, Knape, CC, Webster, HK, Lowder, J, Busch, MP. Lymphocyte subset analysis on frozen whole blood. *Cytometry*. 1997;29:340-350.