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Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections

Please note: This guideline document is obsolete and may not reflect current evidence or best practice and likely contains out-of-date information.

Reported by: Association of State and Territorial Public Health Laboratory Directors and AIDS Program, Center for Infectious Diseases, Public Health Practice Program Office, Centers for Disease Control* The Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) and CDC have collaborated in preparing this report. It includes a description of various interpretive criteria associated with the Western blot test for HIV-1, evaluates the sensitivity and specificity of these criteria as tools for public health practice, and provides recommendations for use of the Western blot and the manner in which to report results in order to provide clinicians and public health policy officials with useful information in their efforts to reach an accurate diagnosis for persons tested for HIV-1 infection. INTRODUCTION

The development of sensitive and specific tests for antibody to human immunodeficiency virus type 1 (HIV-1) progressed rapidly after this retrovirus was identified as the cause of acquired immunodeficiency syndrome (AIDS). These tests have been used for various purposes, including clinical diagnosis of HIV-1 infection--for symptomatic and asymptomatic patients in counseling and testing programs--for seroprevalence surveys, and for blood-donor screening.

Enzyme immunoassay (EIA) is the most widely used serologic test for detecting antibody to HIV-1. Serum samples that are repeatedly reactive in the EIA for HIV-1 antibody are then retested with a supplemental and more specific test, the most common of which is the Western blot (1-3). To date, only one commercial Western blot test (Du PontœPr) has been licensed by the Food and Drug Administration (FDA). The purpose of this report is to provide guidance for interpreting Western blot test results and their use in diagnosing HIV-1 infection. THE WESTERN BLOT ASSAY

The Western blot assay is a method in which individual proteins of an HIV-1 lysate are separated according to size by polyacrylamide gel electrophoresis. The viral proteins are then transferred onto nitrocellulose paper and reacted with the patient's serum. Any HIV antibody from the patient's serum is detected by an antihuman immunoglobulin G (IgG) antibody conjugated with an enzyme that in the presence of substrate will produce a colored band. Positive and negative control serum specimens are run simultaneously to allow identification of viral proteins.

Table 1 lists the major structural proteins coded for by the HIV genome. Antibodies to the HIV-1 major group-specific antigen (GAG) protein p24, and its precursor p55, are the earliest detected after infection by Western blot and tend to decrease or become undetectable with onset or progression of clinical symptoms (4-9). In contrast, antibodies to the envelope (ENV) precursor protein gp160 and the final ENV proteins (gp120 and gp41) can be detected in specimens from virtually all HIV-infected persons regardless of clinical stage (4-9). Antibodies to the polymerase (POL) gene products (p31, p51, and p66) are also commonly detected if these antigens are present on the Western blot strips. However, in a recent study, the protein with a mobility of 160 kilodaltons (kd) present in commercially available Western blots and in viral lysate antigen preparations was identified as a multimer of the gp41 protein (10,11). Furthermore, this study presented evidence that the reaction observed against the gp120 on certain Western blots may have resulted in part from a reaction with a multimeric form of the gp41. In fact, the true gp120 was shown to be absent from some commercial Western blot antigens. When these reagents were used, serum specimens with only gp41 antibodies produced bands at the 41-, 120-, and 160-kd positions. Interpretive Criteria

Although the overall sensitivity and specificity of the Western blot for detection of antibodies to the various viral proteins are high, there has been substantial debate regarding the interpretive criteria. The currently licensed Du Pont Western blot test specifies that the test result should be interpreted as positive only when the detected bands include p24 and p31, and gp41 or gp120/160 (12) (see Table 2). Conversely, a negative Du Pont Western blot test result requires the absence of any and all bands--not just viral-bands. All other patterns are regarded as indeterminate. This interpretation scheme maximizes the specificity of the assay and is mainly intended for use with samples from persons, such as blood donors, for whom there is usually little clinical or virologic information available. (Donated units of blood that are repeatedly reactive by EIA are discarded; Western blot results are used to guide donor notification and deferral.) These criteria are not ideal for all situations, especially the testing of persons at increased risk for HIV infection, or with symptoms suggestive of this infection.

Alternative criteria have been proposed by various groups. ASTPHLD has proposed that a positive test result be defined by the presence of any two of the following bands: p24, gp41, and gp120/160 (13). The Consortium for Retrovirus Serology Standardization (CRSS) has defined a positive test result as the presence of either p24 or p31, plus a diffuse envelope band (i.e., gp41 or gp120/160) (14). The American Red Cross has defined a positive test result as greater than or equal to 1 band from each of the GAG, POL, and ENV gene-product groups (15). These three groups and DuPont all agree that an indeterminate result is the presence of any other band or bands that fail to meet the positive criteria, and that a negative result is the absence of all bands.

The criteria for a negative Western blot interpretation specify "no bands." This interpretation is essential because some observed bands may reflect the presence of antibodies to HIV regulatory proteins or may indicate partially processed or degraded viral structural proteins. Furthermore, different Western blots (commercial, as well as "in-house" preparations) and different virus-antigen preparations used to prepare Western blots may contain different numbers and concentrations of both viral-specific and contaminating cellular proteins that may have unpredictable molecular weights. Evaluation of Criteria

To compare the four sets of criteria for Western blot interpretation, CDC selected 424 serum samples on the basis of the patients' clinical status and EIA results only, and analyzed them using the licensed Du Pont Western blot test (CDC unpublished data). The samples were scored according to each of the criteria (Table 3). For all three categories with repeatedly reactive EIA test results, the Western blot results demonstrate that the ASTPHLD definition gives the highest percentage of positive and the lowest percentage of indeterminate results. The interpretive standards that require the identification of bands from each of the three groups of gene products tend to have indeterminate results for some AIDS and other symptomatic patients due to absence of antibodies to p24 (n=5) or to p31 (n=14) or absence of both types of antibodies (n=2). Since these patients clearly are infected with HIV, the three-gene-product approach to Western blot interpretation is not sensitive enough for public health or clinical practice.

The ASTPHLD/CDC criteria for a positive Western blot differ from the CRSS criteria in two ways: first, ASTPHLD/CDC deletes p31, a change that does not affect the sensitivity or specificity of the criteria (Table 3), and second, ASTPHLD/CDC adds "gp41 and gp120/160," a combination not interpreted as positive with the

CRSS criteria. This latter combination of bands represents antibody to envelope glycoproteins only. In practice, this is a rare finding for asymptomatically infected persons, but it has been reported to be specific for HIV-infected persons and should be included in the positive criteria (9). However, when a Western blot test has only the multimeric form of gp41 and no true gp120 present, a serum sample would be scored as positive on the basis of the presence of antibody to a single envelope glycoprotein, gp41. HIV-1-infected persons with this profile have lost their antibodies to the GAG proteins and are usually symptomatic and do not present a diagnostic problem.

The ASTPHLD/CDC interpretive criteria for a negative result are identical to the FDA recommendation for blood-donor reentry or the Western blot interpretive criteria that are specified in the licensed Western blot kit package insert. RECOMMENDATIONS

On the basis of the results described above, CDC concurred with the ASTPHLD criteria and recommends their use in public health and clinical practice.

Laboratories should report test results as positive, indeterminate, or negative. The Public Health Service recommends that no positive test results be given to clients/patients until a screening test has been repeatedly reactive (i.e., greater than or equal to two tests) on the same specimen and a supplemental, more specific test such as the Western blot has been used to validate those results (3). Upon request, laboratory reports may also contain a list of the bands detected and reference to the interpretive criteria the laboratory uses. Because of the variability of unlicensed reagents, laboratories using non-FDA-licensed Western blots should compare, on a routine basis, their tests with the FDA-licensed Western blot kit using well-characterized serum specimens.

Clinical diagnosis and follow-up of patients is the responsibility of the clinical practitioner. Serologic test results are but one contribution to a patient's data base, which contains medical history (including high-risk behavior or exposure to HIV), results of physical examination, and other clinical findings. Clinicians must consider the total profile for a client when attempting to make a diagnosis after indeterminate Western blot results have been obtained. Accurate diagnosis for such persons can be challenging--and the challenge can be complicated by the tendency of some clients to become distressed by the apparent "uncertainty" of their test results.

Clinical follow-up of patients with indeterminate Western blot results may require many months of observation, interviewing, and testing. Most indeterminate patterns involve p18 (also referred to as p17), p24, or p55, or any combination of these three proteins (16-18). In one study of 390 "atypical" or indeterminate samples, 53% reacted against p24, with or without p18 or p55; 47% reacted against p18 (but not p24), with or without p55 (18).

Some indeterminate results may be obtained with serum samples from persons who are in the process of seroconverting. A compilation of 209 volunteer blood donors with GAG-only indeterminate Western blot results were followed for as long as 2 years (17-21). During that time, only five of 134 persons who had initially reacted to p24 developed additional bands on the Western blot test. None of the 75 persons who initially reacted against p18 (but not p24) developed additional bands. The five persons who did seroconvert had positive results when their first follow-up samples were tested. The intervals between initial and follow-up tests were 8 weeks (two persons), 20 weeks (two persons), and 32 weeks (one person). The three longest intervals reflected delays in follow-up testing and not the actual time to seroconversion. These results do not refute earlier findings that seroconversion typically occurs within 3 months of infection (5,22). The importance of careful risk assessment for persons with indeterminate Western blot patterns was reemphasized when in one study (18) two of three people who initially had indeterminate results (but later seroconverted) disclosed histories of risk behavior when they were reinterviewed during follow-up.

A person whose Western blot test results continue to be consistently indeterminate for at least 6 months--in the absence of any known risk factors, clinical symptoms, or other findings--may be considered to be negative for antibodies to HIV-1. Such persons should be reassured that they are almost certainly not infected with HIV-1. However, no large-scale studies have been done to provide virologic data to confirm independently the serologic findings from the studies of clients whose Western blot test results are consistently indeterminate. In contrast, an asymptomatic person who has an indeterminate Western blot test result and a history of possible exposure to or

symptoms compatible with HIV infection requires additional diagnostic follow-up. This should include conducting serial Western blot testing, assessing the function of the individual's immune system, and eliciting the cooperation of the person's sexual and needle-sharing partners to determine whether they are infected. Individuals with a pattern of indeterminate Western blot test results should not donate blood or plasma for either transfusion or use in manufactured blood products.

As the HIV/AIDS epidemic continues, additional tests of higher specificity will be needed to decrease the number of false-positive reactions and to permit correct diagnosis of HIV infection in a larger spectrum of clinical situations in which an indeterminate antibody profile exists. The use of new antibody tests based on antigens derived by recombinant deoxyribonucleic acid (DNA) technology or the application of DNA probe technology--particularly DNA amplification by the polymerase chain reaction (PCR)--already shows promise in this area (23).

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