EDUCATIONAL COMMENTARY – HUMAN IMMUNODEFICIENCY VIRUS: NEW LABORATORY TESTING AND PROPOSED ALGORITHM FOR DIAGNOSIS

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**Florida licensees, please note: This exercise is NOT intended to fulfill your state education requirement for HIV/AIDS.

LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- identify patients who should be tested for human immunodeficiency virus (HIV).
- describe the new proposed testing algorithm for HIV diagnosis.
- explain the advantages and disadvantages of the new proposed algorithm for HIV diagnosis.
- discuss the diagnostic use of the Western blot, the third-generation enzyme immunoassay, the fourth-generation antibody/antigen immunoassay, and nucleic acid testing.
- explain the reasons for differentiation of HIV-1 from HIV-2.

Human immunodeficiency virus (HIV) continues to be a major public health problem in the United States. The Centers for Disease Control and Prevention (CDC) estimates that more than 1.1 million people in the United States are living with HIV infection, and of these, almost 1 in 5 (18.1%) are unaware of their current infection.¹ The estimated incidence of HIV has remained stable the past few years at about 50,000 new HIV infections per year.¹

Increasing the number of persons who are aware of their HIV-positive status is critical for preventing HIV transmission. To reduce HIV infections and improve the health of patients with HIV infection through early diagnosis, the CDC has updated its recommendations for HIV testing. The Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings,² published by the CDC in 2006, promotes HIV screening in health care settings among all persons aged 13 through 64 years. Yearly screening for those at high risk for infection and screening as a routine part of prenatal care for all pregnant women are among the recommendations. The purpose of the Revised Recommendations is to promote HIV screening as routine medical care similar to cholesterol screening. When these recommendations are appropriately implemented, it is possible to diagnose infection earlier, to link infected persons to medical care, to ensure that prevention services are provided to infected patients, and to reduce the number of perinatal transmissions.

In 1989, the Association of Public Health Laboratories (APHL) in collaboration with the CDC created the original HIV testing algorithm, which called for a specimen repeatedly reactive by a screening
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immunoassay to be confirmed by a supplemental HIV antibody test, either a Western blot or indirect immunofluorescence assay. Since the development of the first HIV testing algorithm, HIV testing and technology have changed significantly. New immunoassays, point-of-care rapid tests, and molecular detection assays have recently been introduced. When used together in an appropriate testing algorithm, these tests can identify the greatest number of new HIV cases.

During the past several years, the Association of Public Health Laboratories and the CDC have gathered data to support a new testing algorithm. In 2009, the two organizations released a document, HIV Testing Algorithms: A Status Report, which described several HIV testing algorithms that had the potential to augment and provide alternatives to the original testing algorithm. Between 2008 and 2011, the CDC convened several work groups to consider revising various aspects of HIV surveillance, including adopting new HIV diagnostic testing algorithms, criteria for differentiating HIV-2 infection from HIV-1 infection, and expanding available testing to include acute HIV infection. The two organizations next came together at the 2010 HIV Diagnostics Conference, where a new HIV algorithm was proposed. In February 2011, APHL held a series of conference calls to focus on new trends in HIV diagnostics, including the benefits of and barriers to the new proposed algorithm.

In June 2011, the Clinical and Laboratory Standards Institute (CLSI) published Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection. This document provides guidance for laboratory professionals who perform HIV testing and for health care professionals who interpret the results. This document comprises multiple algorithms addressing every scenario an institution may encounter. The first algorithm presented is the new proposed algorithm (Figure I) from the APHL and CDC.

The new proposed algorithm calls for an initial screening test of an antigen-antibody combination assay, or a fourth-generation test. If repeatedly reactive, it is followed by an immunoglobulin (Ig) G antibody immunoassay that differentiates HIV-1 from HIV-2 antibodies. Specimens negative for HIV-1 and HIV-2 antibodies by the differentiation assay are tested for HIV-1 RNA using nucleic acid testing (NAAT). The new algorithm is designed to produce a high positive predictive value using a minimum of tests, with the ultimate goal of identifying more infections earlier and differentiating HIV-1 from HIV-2.

The first step in the proposed algorithm is the recommendation of an antibody-antigen combination immunoassay. These fourth-generation tests detect HIV-1 groups M, N, and O and HIV-2 antibodies. In addition, they detect the p24 antigen of HIV. The major advantage of the fourth-generation assays over the third-generation antibody enzyme immunoassays is the elimination of the “window period,” during which the infected person has not seroconverted. This earlier detection is a result of the fourth-
Figure I.

**APHL/CDC - Proposed HIV Testing Algorithm**

- **HIV-1/HIV-2 Ag/Ab Immunoassay**
  - (+) Repeat testing x2
  - (-) Negative for HIV-1 and HIV-2 antibodies and p24 antigen
  - (+/-) or (+/-) HIV-1 / HIV-2 Ab Differentiation Immunoassay
    - HIV-1 (+) HIV-2 (-) Positive for HIV-1 Ab
    - HIV-1 (-) HIV-2 (+) Positive for HIV-2 Ab
    - HIV-1 (+) HIV-2 (+) Positive for HIV Ab #
    - Key
      - *An IgM - Sensitive Ab Immunoassay can be used if the Ag / Ab combination immunoassay is not available*
      - # HIV-Ab positive; Further testing is required to rule out dual infection
      - & Consider HIV-2 DNA testing if indicated

*Used with permission from the Association of Public Health Laboratories (APHL)*

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generation assay’s ability to detect p24 antigen. The p24 antigen can be detected as early as, and in some cases earlier than, 17 days after infection with HIV and up to 20 days sooner than third-generation HIV antibody enzyme immunoassays, which detect only antibodies to HIV. Early detection of HIV infection is crucial to decreasing transmission, because the risk for transmission from persons with early infection is much higher than from persons with established infection. This is owing not only to the high viral load, but also to the presence of variants that are more capable of causing infection during the acute phase. There are currently two Food and Drug Administration (FDA)-approved fourth-generation immunoassays available for HIV testing. These include the ARCHITECT HIV Ag/Ab Combo Assay (Abbott Laboratories) and the GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories). Overall, the fourth-generation antigen-antibody assays have specificity and sensitivity comparable to those of third-generation antibody-only assays.

If the screening antibody-antigen combination immunoassay is repeatedly reactive, the next step in the proposed algorithm is to differentiate HIV-1 from HIV-2 antibodies. A test that discriminates between HIV-1 and HIV-2 is needed because HIV-2 infections require different clinical management and are less likely to progress to AIDS. Although uncommon in the United States, cases of HIV-2 continue to be confirmed and have been reported in at least 17 states in the past 3 years, including in low-prevalence areas. The CDC also recommends differentiation of HIV because HIV-2 does not respond to certain first-line antiretroviral drugs commonly used to treat HIV-1 infections and NAAT for detection of HIV-1 cannot effectively detect or monitor HIV-2 infections. In the United States, the Multispot (Bio-Rad) is the only FDA-approved assay that can discriminate between HIV-1 and HIV-2 antibodies.

The proposed algorithm eliminates the traditional confirmatory Western blot assay as the second step for several reasons. First, the advancements of the third-generation enzyme immunoassays enabled the detection of IgG- and IgM-class antibodies. The Western blot, although very specific, detects only IgG antibodies. Second, the fourth-generation antigen-antibody immunoassays for the detection of HIV have greater sensitivity. A third- or fourth-generation assay can show a reaction up to 3 weeks before a confirmatory Western blot test would produce a positive result. Third, HIV-2 may be misdiagnosed as HIV-1 owing to extensive cross-reactivity on the Western blot. The Western blot is highly regarded for its specificity when positive, but indeterminate results may be encountered for several reasons. These include an HIV-2 infection, HIV vaccination, cross-reactivity with other viruses, acute HIV infection with incomplete seroconversion, late-stage HIV infection (AIDS), and other infections. If a result is indeterminate by Western blot, it must be followed up with additional testing. Finally, the one FDA-approved HIV-1/HIV-2 discriminatory assay on the market is a rapid antibody test that can be performed by most clinical laboratories. This test is slightly more sensitive than the Western blot and can be
performed in as little as 15 minutes. Overall, the elimination of the Western blot allows for earlier diagnosis of HIV.

Within the proposed algorithm, specimens negative for HIV-1 and HIV-2 antibodies by the differentiation assay are tested for HIV-1 RNA. This NAAT detects HIV RNA that may be present during the window period between infection and the development of detectable antibodies. This is the first algorithm that has recommended the use of NAAT for diagnosis of HIV. The NAAT is the most sensitive assay for detection of HIV infection. Although it is included as the final step for inconclusive results in the new algorithm, there is currently only one FDA-approved NAAT available for diagnostic use. The APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe) is approved for confirmation of repeatedly reactive immunoassay results and for the diagnosis of acute HIV infection.

There are pros and cons associated with the new APHL/CDC-proposed HIV-testing algorithm. Benefits include earlier detection of acute HIV infections, differentiation of HIV-1 from HIV-2, elimination of indeterminate and inconclusive results, decreased technologist time and potential cost savings, and same-day reporting of results to facilitate initiation of care. Limitations of the new algorithm include the small number of testing options currently available and approved by the FDA, and the cost of fourth-generation screening platforms. There will also be a need for a second algorithm for HIV testing using oral fluid and dried blood spots as specimens. Last, the low-volume of specimens that will require NAAT will challenge many laboratories to maintain a low-volume assay or send HIV NAATs to a reference laboratory.

Of note, the proposed algorithm and CLSI document M53-A are intended for use in the laboratory diagnosis of HIV infection in the health care setting and do not address methods or strategies for screening the blood supply or for organ or tissue donation. The algorithm is not intended for use outside the clinical setting. The CLSI M53-A criteria are primarily intended for advanced diagnostic laboratories and point-of-care-settings. The recommended algorithms vary based on the laboratory setting and the population it serves. Each public health, clinical, and commercial laboratory must select the specific tests and testing strategies that are most feasible and appropriate for its circumstances.

References


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