THE LABORATORY DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV): Clinical Considerations from the Department of Veterans Affairs National HIV Program, in the HIV, Hepatitis and Public Health Pathogens Program, Office of Patient Care Services

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Please send questions or comments about this document to the Director, HHPHP, by email at VHAHHPHP@va.gov

PURPOSE

These clinical considerations provide updated recommendations for the laboratory diagnosis of Human Immunodeficiency Virus (HIV) and include current scientific evidence for the use of rapid testing for HIV, including performance characteristics of rapid tests relative to traditional HIV tests. These clinical considerations assist clinicians and Laboratory Directors on compliance with applicable Federal and VHA laboratory regulatory standards. Clinicians and facility Laboratory Directors may also use this document when considering the implementation of rapid HIV testing.

BACKGROUND

Undiagnosed HIV infection is common in the United States (U.S.); the Centers for Disease Control and Prevention (CDC) estimates that approximately 14 percent of those infected with HIV are unaware of their diagnosis. Since these individuals have not been linked to medical care and antiretroviral therapy, they are at increased risk of HIV-related complications; in addition, these individuals are 3.5 times more likely to unknowingly sexually transmit HIV than patients who are aware of their diagnosis.

In 2006, CDC recommended voluntary HIV testing of patients aged 13-64 years in all health care settings unless the diagnostic yield is less than 0.1 percent. In accordance with CDC recommendations, on August 17, 2009, VHA changed its policy on HIV testing from “risk-based” screening to routine voluntary HIV testing of all adult Veterans as a part of routine medical care.

The previous testing algorithm for diagnosing HIV in the U.S. started with screening with an immunoassay (IA). If the immunoassay was reactive, a confirmatory test such as the Western blot or the indirect immunoassay (IFA) was then performed. However, this diagnostic algorithm yielded false negative or indeterminate results early in HIV infection, and the confirmatory HIV-1 Western Blot misclassified approximately 46-85 percent of HIV-2 infections as HIV-1.

As a result, on June 27, 2014, CDC released updated recommendations for laboratory testing for the diagnosis of HIV infection using serum or plasma specimens (Attachment A). The CDC now recommends that laboratories conduct initial testing for HIV with an FDA-approved antigen/antibody combination immunoassay that detects HIV-1 and HIV-
2 antibodies and HIV-1 p24 antigen (frequently referred to as a "4th generation test")\(^5\). Non-reactive specimens undergo no further testing. Reactive specimens then undergo testing with an immunoassay that differentiates HIV-1 from HIV-2 antibodies (an HIV-1/HIV-2 antibody differentiation assay). A reactive antigen/antibody combination assay followed by a reactive HIV-1/HIV-2 antibody differentiation assay is considered a positive HIV result. In cases where the initial combination immunoassay is reactive and the subsequent HIV-1/HIV-2 antibody differentiation assay is non-reactive or indeterminate, HIV RNA testing is then performed\(^6\).

The U.S. Food and Drug Administration (FDA) has approved one rapid HIV test for use on oral fluid, which is the OraQuick Advance Rapid\(^6\). Other FDA-approved rapid HIV tests must be performed on whole blood (obtained by venipuncture or finger-stick), serum or plasm. Rapid tests fall into various categories under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88): waived, moderate complexity, or high complexity. There are currently seven CLIA- waived rapid HIV tests suitable for use in clinical and non-clinical settings, and ten CLIA – waived moderate complexity rapid HIV tests suitable for laboratory use\(^6,7\).

Reactive rapid HIV tests are considered preliminary positives and the results must be confirmed with supplemental testing. The CDC recommends that the algorithm outlined below (Attachment A) be used for any reactive rapid HIV test result, beginning with a laboratory-based antigen/antibody combination immunoassay using a serum or plasma specimen. If the laboratory-based antigen/antibody combination immunoassay is non-reactive, no further testing is recommended and the rapid test is considered a false positive. If the laboratory-based combination immunoassay is positive, the HIV-1/HIV-2 antibody differentiation assay is performed\(^4\).

The sensitivity and specificity of rapid HIV tests is greater than 99.5 percent, comparable to conventional tests processed in the laboratory\(^8\). As with all screening tests, lower positive predictive values are seen in areas of low prevalence. However, the negative predictive value of rapid tests is greater than 99.9 percent.

**RECOMMENDATIONS**

In accordance with CDC and VHA’s National Center for Health Promotion and Disease Prevention\(^9\) recommendations, VHA laboratories are encouraged to update their testing algorithm and testing technologies to the following:

- VHA laboratories should follow the CDC-recommended testing algorithm (found below)

- Initial laboratory testing for the diagnosis of HIV infection should rely on serum or plasma specimens using an FDA-approved antigen/antibody combination immunoassay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen (a "4th generation" test). Reactive specimens should undergo testing with an immunoassay that differentiates HIV-1 from HIV-2 antibodies (an HIV-1/HIV-2 antibody differentiation assay).
VA medical facilities are encouraged to use FDA-approved rapid HIV tests, particularly in clinical environments or for demographic groups where linkage to medical care is difficult (e.g., homeless clinics, substance use clinics, rural clinics, women’s clinics, and Emergency Departments).

All health care providers can perform a rapid HIV test as long as they are authorized to do so under their local facility scope of practice, have received appropriate documented training, and meet other applicable regulatory requirements as determined by the facility Laboratory Director.

Individuals involved in implementing rapid HIV testing must work closely with Laboratory Directors prior to the implementation of any rapid HIV testing program to ensure the testing program is implemented in compliance with VA policies. Under VHA Directive 1113 (http://www.va.gov/vhapublications/ViewPublication.asp?pub_ID=3104z), Laboratory Directors are responsible for compliance with Federal and VA policies with respect to CLIA-88 and VHA Handbook 1106.01 (http://www.va.gov/vhapublications/ViewPublication.asp?pub_ID=3104z); therefore, clinical providers performing point-of-care rapid HIV testing need to comply with applicable policies put forth by the Laboratory Director. Laboratory Directors are encouraged to support efforts to expand HIV rapid testing.
RECOMMENDED LABORATORY HIV TESTING ALGORITHM FOR SERUM OR PLASMA SPECIMENS

**HIV-1/2 antigen/antibody combination immunoassay**

1. **(+)**
   - HIV-1/2 antibody differentiation immunoassay

2. **(-)**
   - Negative for HIV-1 and HIV-2 antibodies and p24 Ag

**HIV-1/HIV-2 antibody differentiation immunoassay**

1. HIV-1 (+)
   - HIV-1 antibodies detected
   - HIV-1 NAT (+)
   - Acute HIV-1 Infection
2. HIV-1 (-)
   - HIV-2 (+)
   - HIV-2 antibodies detected
   - HIV-1 NAT (-)
   - Negative for HIV-1
3. HIV-1 (+)
   - HIV antibodies detected
   - HIV-1 NAT
4. HIV-1 (-) or indeterminate
   - HIV-2 (-)

1) Laboratories should conduct initial testing for HIV with an FDA-approved antigen/antibody combination (4th generation) immunoassay* that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to screen for established infection with HIV-1 or HIV-2 and for acute HIV-1 infection. No further testing is required for specimens that are nonreactive on the initial immunoassay.

2) Specimens with a reactive antigen/antibody combination immunoassay result (or repeatedly reactive, if repeat testing is recommended by the manufacturer or required by regulatory authorities) should be tested with an FDA-approved antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies. Reactive results on the initial antigen/antibody combination immunoassay and the HIV-1/HIV-2 antibody differentiation immunoassay should be interpreted as positive for HIV-1 antibodies, HIV-2 antibodies, or HIV antibodies, undifferentiated.

3) Specimens that are reactive on the initial antigen/antibody combination immunoassay and nonreactive or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with an FDA-approved HIV-1 nucleic acid test (NAT).
a. A reactive HIV-1 NAT result and nonreactive HIV-1/HIV-2 antibody differentiation immunoassay result indicates laboratory evidence for acute HIV-1 infection.

b. A reactive HIV-1 NAT result and indeterminate HIV-1/HIV-2 antibody differentiation immunoassay result indicates the presence of HIV-1 infection confirmed by HIV-1 NAT.

c. A negative HIV-1 NAT result and nonreactive or indeterminate HIV-1/HIV-2 antibody differentiation immunoassay result indicates a false-positive result on the initial immunoassay.

4) Laboratories should use this same testing algorithm, beginning with an antigen/antibody combination immunoassay, with serum or plasma specimens submitted for testing after a reactive (preliminary positive) result from any rapid HIV test.


* Exception: As of April 2014, data are insufficient to recommend use of the FDA-approved single-use rapid HIV-1/HIV-2 antigen/antibody combination immunoassay as the initial assay in the algorithm. Positive results from such assays (e.g., at a homeless Veteran stand down testing program) should be confirmed using laboratory-based 4th-generation assays.

REFERENCES

2. Marks G, Crepaz N, Janssen RS. “Estimating sexual transmission of HIV from persons who are unaware and aware that they are infected with the virus in the USA.” AIDS. 2006; 20:1447--50.
