NEW YORK
state department of
HEALTH

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Commissioner

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Executive Deputy Commissioner

September 5, 2013

RE: Information for Clinicians on a New Diagnostic Testing Algorithm for Human Immunodeficiency Virus (HIV) Infection

Dear Colleague:

The purpose of this letter is to update clinicians about a new HIV Diagnostic Testing Algorithm for diagnosis of HIV infection. In the past, HIV diagnostic testing was based solely on antibody detection and tests such as Western blot or IFA (immunofluorescent antibody) were required to confirm HIV antibodies following a reactive screening test. While these traditional confirmatory tests continue to have a role in HIV diagnosis, technological developments over the past few years have allowed assessment of testing algorithms that do not rely on these methods. A new HIV Diagnostic Testing Algorithm has been verified through scientific studies[1] and offers several advantages over the traditional enzyme immunoassay (EIA)-Western blot algorithm, including earlier and more accurate detection of HIV infections, the ability to differentiate between HIV-1 and HIV-2 infections, and lower costs.[2,3,4] Recommendations from the Centers for Disease Control and Prevention (CDC) for its use are anticipated. The New York State Department of Health (NYSDOH) recently provided interim guidance to NYS-permitted laboratories, and this letter provides information for clinicians on use of the new algorithm.[5]

Two attachments accompany this letter:
- Attachment 1 The HIV Diagnostic Testing Algorithm
- Attachment 2 Interpreting Clinical Laboratory Results from the HIV Diagnostic Testing Algorithm

New Laboratory Algorithm

The new HIV Diagnostic Testing Algorithm is described in **Attachment 1**. This algorithm differs significantly from the conventional strategy of antibody screening followed by the Western blot. The HIV Diagnostic Testing Algorithm is a multi-test algorithm, incorporating tests that detect HIV antigens, antibodies and RNA, and the final interpretation is based on a combination of test results rather than a single confirmatory test such as the Western blot.

An important factor in HIV diagnostic testing is the sensitivity of the initial test. The new algorithm is intended to begin with an HIV-1/2 immunoassay capable of detecting HIV antigens (Ag) and antibodies (Ab), commonly referred to as an Ag/Ab combo or 4th generation immunoassay. The benefit of this technology is that it can detect HIV-1 during the acute stage via antigen detection as well as chronic stages of infection via antibody detection. When the 4th generation test result is 'Reactive' this is considered to be a presumptive positive result and additional testing is required for confirmation. In the new algorithm, confirmation is provided by a specific sequence of supplemental tests. The first is a supplemental test that has been FDA-approved to detect and differentiate HIV-1 and HIV-2 antibodies. If this test is positive for HIV-1 antibodies or HIV-2 antibodies, HIV antibodies are confirmed, and clinicians may proceed with tests appropriate for initial evaluation of an infected individual. If HIV antibodies are not confirmed, the laboratory should reflex to an HIV-1 RNA (nucleic acid test or NAT) detection test to distinguish between a false positive

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screening result and an acute HIV infection. If the RNA test is positive, HIV-1 infection is present and the patient is likely to be in the acute or very early stage of infection.

Testing Reports for Health Care Providers

The HIV Diagnostic Testing Algorithm includes combinations of HIV test results that may be unfamiliar to health care providers. Therefore, in addition to the results of all tests, the laboratory report that is returned to the ordering clinician should include a final interpretation statement and, when appropriate, recommendations for follow-up testing. A list of the different combinations of test results and the NYSDOH recommended interpretation statements is provided in **Attachment 2**. Providers may request that preliminary HIV test results be reported to them prior to completion of the algorithm. Additional information on currently available tests appropriate for the algorithm may be found at: http://www.health.ny.gov/diseases/aids/testing/index.htm#algorithm/.

Implications for Testing

Alternatives to 4th generation immunoassays: 3rd generation HIV-1/2 immunoassays are capable of detecting IgM and IgG antibodies to HIV and have been used for HIV screening by laboratories for several years. Some laboratories may continue to use 3rd generation immunoassays as their initial screening test, and it is currently acceptable for laboratories to follow the HIV Diagnostic Testing algorithm with a 3rd generation immunoassay as the initial test[1]. Clinicians should be aware that 3rd generation immunoassays will detect infection early in the seroconversion process, even before a Western blot becomes positive, but they will not detect HIV-1 infection in the acute stage before antibodies are produced. The test results and interpretations in **Attachment 2** are based on initial testing with a 4th generation HIV-1/2 Ag/Ab combo immunoassay. If the initial test in the algorithm is a 3rd generation HIV-1/2 immunoassay, the interpretation statements will be slightly different.

Rapid Point-of-Care Testing: Rapid testing continues to be an approved method for HIV screening, and an important means for providing access to HIV testing, especially in community-based settings. Health care providers and community based organizations that hold a NYSDOH Limited Service Laboratory Registration to perform rapid HIV testing, as well as licensed clinical laboratories, may continue to screen for HIV using FDA-approved rapid tests. All preliminary positive HIV rapid test results must be confirmed through additional testing performed at a clinical laboratory, and the HIV Diagnostic Testing Algorithm may be used in place of the Western blot for this purpose. Clinical laboratories have received instructions on how to use the HIV Diagnostic Testing Algorithm when confirming a rapid test result and will receive updates as new FDA-approved tests become available.

Use of Western blot: FDA-approved HIV-1 Western blot tests are still available and may be used by laboratories to confirm reactive screening test results; however, the HIV-1 Western blot tests are less sensitive than most available HIV screening tests, including rapid tests. A negative Western blot result should not be interpreted as negative for HIV-1 infection. If the Western blot result is negative or indeterminate, an HIV-1 RNA test should be performed on the specimen to resolve the discrepancy. If the laboratory is unable to perform an HIV-1 RNA test directly on the original specimen, the laboratory test report should include a statement recommending collection of a specimen for HIV-1 RNA testing as soon as possible.

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Recent HIV Exposure: Patients with potential recent exposure to HIV present diagnostic challenges due to the "window period," or the length of time after infection that it takes for the virus to become detectable by HIV diagnostic tests. The length of the window period varies depending on the type of diagnostic test used and the method it employs to detect the virus. More information about the window period for various types of tests can be found at http://www.hivguidelines.org/clinical-education/hiv-education-and-training-initiative/hiv-qa-fact-sheets/window-period-for-hiv-infection/. Clinicians should be familiar with the testing process used by the laboratory conducting testing for their patients, as recommendations for retesting patients with recent exposure will vary depending on the test used.

Clinical Assessment: This testing algorithm is expected to perform as well as Western blot testing for the diagnosis of chronic HIV infection. However, as with Western blot testing, occasional persons may have test results that are confusing or appear inconsistent with the clinical presentation. Consultation with an HIV clinical expert is appropriate if test results conflict with patient history and/or clinical presentation.

HIV-2 Evaluation: There are no FDA-approved HIV-2 RNA or DNA tests currently available. A laboratory-developed HIV-2 RNA test that has been approved for diagnostic use is available at the NYSDOH Wadsworth Center. If HIV-2 RNA testing is warranted, contact the Wadsworth Center at (518) 474-2163.

HIV Reporting

Public Health Law Article 21 (Chapter 163 of the Laws of 1998) requires the reporting of persons with HIV as well as AIDS to the NYSDOH within 14 days of diagnosis. The law also requires that reports contain the names of sexual or needle-sharing partners known to the medical provider or whom the infected person wishes to have notified. A NYSDOH reporting form, the Medical Provider Report Form (DOH-4189) must be completed for persons with the following diagnoses:

1. **Initial/New HIV diagnosis** - First report of testing documenting HIV infection.
2. **Previously diagnosed HIV infection (non-AIDS)** - Infection previously diagnosed (including repeat/confirmatory test) but patient has not met criteria for AIDS. (Applies to a medical provider who is seeing the patient for the first time.)
3. **Initial/New Diagnosis of AIDS** - Including <200 CD4 cells/µL or opportunistic infection (AIDS-defining illness).
4. **Previously diagnosed AIDS** - (Applies to a medical provider who is seeing the patient for the first time.)

Blank forms are available by calling the NYSDOH (518) 474-4284. The NYSDOH Bureau of HIV/AIDS Epidemiology and the New York City Department of Health and Mental Hygiene HIV Surveillance and Field Services Program will also work with clinicians to understand the documentation needed for reporting of HIV and AIDS diagnoses as required by Public Health Law 2130.

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Additional Information

As it becomes available, additional information and guidance on the new HIV Diagnostic Testing Algorithm will be posted on the NYSDOH website under "HIV Testing". Questions may be directed to hivtesting@health.state.ny.us. The new HIV Diagnostic Testing Algorithm offers the opportunity to provide infected persons and their clinicians more accurate and timely information on HIV-1 and HIV-2 infection as well as the opportunity to improve capacity to detect acute HIV infection. We look forward to collaborating with you in continuing the important work of prevention, diagnosis and treatment of HIV infection by incorporating the HIV Diagnostic Testing Algorithm into routine diagnostic testing and HIV surveillance.

Sincerely,

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References
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2.   CLSI. Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline. CLSI Document M-53A, 2011.
3.   Branson, BM. The Future of HIV Testing. J Acquir Immune Defic Syndr 2010;55:S102-S105.
4.   Centers for Disease Control and Prevention (CDC). Detection of Acute HIV Infection in Two Evaluations of a New HIV Diagnostic Testing Algorithm – United States, 2011-2013. MMWR 2013; 62 (24); 489-494.
5.   NYSDOH. "Interim Guidelines for Laboratories on the use of a new Diagnostic Testing Algorithm for Human Immunodeficiency Virus (HIV) Infection"; http://www.hivguidelines.org/wp-content/uploads/2013/05/interim-guidelines-for-laboratories-on-the-use-of-a-new-diagnostic-testing-algorithm-for-hiv-infection-05-16-2013.pdf. Accessed June 12, 2013.

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Attachment 1

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HIV Diagnostic Testing Algorithm

Step 1. HIV-1/2 Ag/Ab combo immunoassay (4th generation)

(+) → **(−)**
Negative for HIV-1 and HIV-2 antibodies and HIV-1 p24 Ag*

Step 2. HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)	HIV-1 (−)	HIV-1 (+)	HIV-1 (−) or indeterminate
HIV-2 (−)	HIV-2 (+)	HIV-2 (+)	HIV-2 (−)
Positive for HIV-1 antibodies	*Positive for HIV-2 antibodies*	**(Undifferentiated)** *Positive for HIV antibodies*	

Step 3. HIV-1 RNA assay

RNA (+)
Positive for HIV-1

RNA (−)
Negative for HIV-1

(+) = Reactive (or repeatedly reactive) test result, in accordance with manufacturer's instructions
(−) = Nonreactive test result, in accordance with manufacturer's instructions
Italics = Final interpretation; No further testing indicated for the specimen
*For 3rd generation HIV-1/2 immunoassay, interpretation is 'Negative for HIV-1 and HIV-2 antibodies'.

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Step 1. **4th generation HIV-1/2 Ag/Ab combo immunoassay (preferred) or 3rd generation HIV-1/2 immunoassay (acceptable)**. If the result from this test is 'Nonreactive', no further testing of the specimen is indicated. If the result is 'Reactive', this is considered to be a preliminary positive result and supplemental testing must be performed, beginning with an HIV-1/HIV-2 antibody differentiation immunoassay (step 2).

Step 2. HIV-1/HIV-2 antibody differentiation immunoassay. If the initial HIV-1/2 immunoassay (step 1) was reactive and the result of HIV-1/HIV-2 antibody differentiation immunoassay is 'Reactive' for HIV-1 or HIV-2 antibodies, the interpretation is 'Positive for HIV-1 antibodies' or 'Positive for HIV-2 antibodies', respectively. No further testing of the specimen is required and medical care is recommended. If the result is 'Reactive' for both HIV-1 and HIV-2 antibodies (i.e. HIV Positive, Undifferentiated), the interpretation is 'Positive for HIV antibodies' and medical care is recommended. Additional testing for HIV-1 RNA and HIV-2 RNA or DNA is recommended at the initial clinical evaluation to verify or rule-out HIV-1/HIV-2 dual infection. If the result of the HIV-1/HIV-2 antibody differentiation test is 'Nonreactive' or 'Indeterminate', testing of the specimen should reflex to an HIV-1 RNA assay (step 3).

Step 3. HIV-1 RNA assay. If the initial HIV-1/2 immunoassay (step 1) was reactive and HIV-1 RNA is detected, the final interpretation is 'Positive for HIV-1' and medical care should be initiated. If HIV-1 RNA is not detected, the final interpretation is 'Negative for HIV-1'. The initial HIV-1/2 immunoassay result was most likely a false positive. If there is reason to suspect recent HIV-2 infection, follow-up testing for HIV-2 RNA or DNA should be considered.

September 5, 2013

Attachment 2

Interpreting Clinical Laboratory Results from the HIV Diagnostic Testing Algorithm

	Test Method	Test Result	Interpretation
A	1. HIV-1/2 Ag/Ab combo immunoassay	1. Nonreactive	Negative for HIV-1 antigen and HIV-1/HIV-2 antibodies. No laboratory evidence of HIV infection.
B	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay	1. Reactive 2. HIV-1 Positive	Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present. *Provider case reporting required**
C	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay	1. Reactive 2. HIV-2 Positive	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present. *Provider case reporting required**
D	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay 3. HIV-1 RNA assay	1. Reactive 2. Nonreactive or Indeterminate 3. Not detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected. No laboratory evidence of HIV-1 infection is present. Further testing is recommended if warranted by clinical evaluation or risk factors.
E	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay 3. HIV-1 RNA assay	1. Reactive 2. Nonreactive or Indeterminate 3. Detected	Positive for HIV-1. Laboratory evidence of HIV acute HIV-1 infection is present. *Provider case reporting required**
F	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay	1. Reactive 2. HIV Positive (Undifferentiated)	Positive for HIV antibodies. Laboratory evidence of HIV infection is present. HIV antibodies could not be differentiated as HIV-1 or HIV-2. Additional testing for HIV-1 RNA and HIV-2 RNA or DNA is warranted. *Provider case reporting required**
G	1. HIV-1/2 Ag/Ab combo immunoassay 2. HIV-1/HIV-2 antibody differentiation immunoassay	1. Reactive 2. Nonreactive or Indeterminate	HIV-1 antibodies were not confirmed and HIV-1 RNA testing was not performed. **Testing of this specimen is incomplete. Follow-up testing for HIV antibodies and HIV-1 RNA is recommended as soon as possible.**

*Provider case reporting required: Under New York State public health law, medical providers are required to report to the NYSDOH cases of HIV infection, HIV-related illness, AIDS and, for newly diagnosed cases, the names of all contacts known to the provide using the NYS Medical Provider HIV/AIDS and Partner/Contact Report Form (PRF) (DOH-4189 revised 8/05) **within 14 days of diagnosis**. Please contact the NYSDOH at (518) 474-4284 for additional information and reporting forms. In New York City, contact NYCDOHMH at 212-442-3388.

September 5, 2013