Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm

November 2013

APHL ASSOCIATION OF
PUBLIC HEALTH LABORATORIES ™
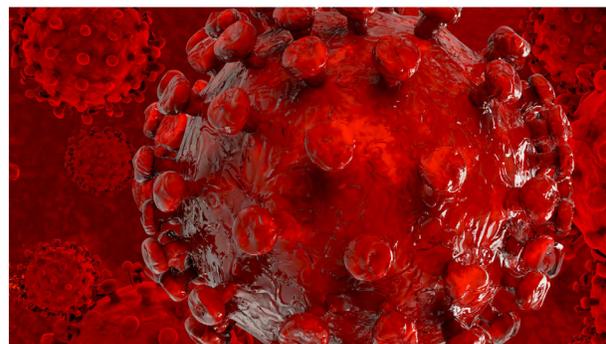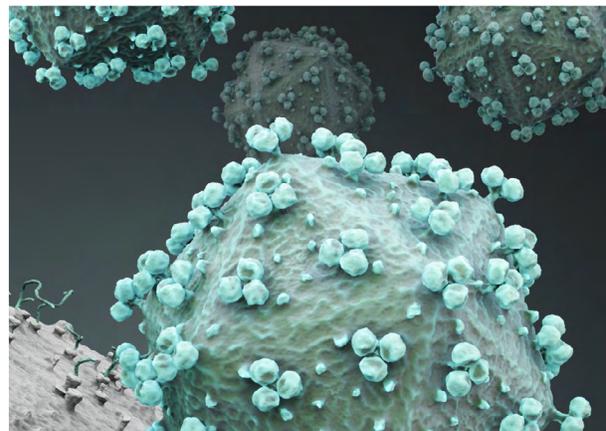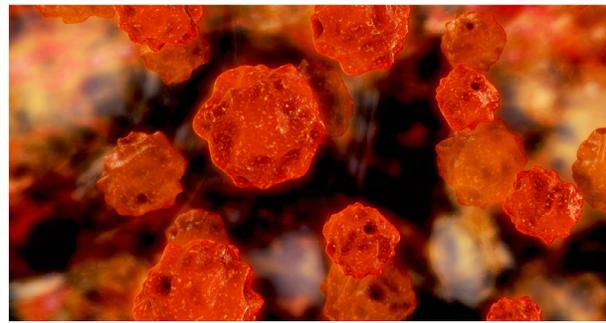
Introduction

In March 2010, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) proposed a new HIV Laboratory Diagnostic Testing Algorithm which was updated at the December 2012 HIV Diagnostics Conference (Figure 1).[1] The algorithm is presented as one of the options in the Clinical Laboratory Standards Institute (CLSI) guideline document M53-A entitled "Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection"[2]* and the updated version is described in CDC's "Draft Recommendations: Diagnostic Laboratory Testing for HIV Infection in the United States."[3] The revised algorithm recommends initial testing with a 4th generation HIV-1/2 antigen/antibody combination immunoassay (IA) which, if reactive, is followed by supplemental testing with an HIV-1/2 antibody differentiation assay. Specimens negative or indeterminate by the HIV-1/2 antibody differentiation assay undergo an HIV-1 nucleic acid test (NAT). This marks the first time since 1989 that Western blot was not included in the HIV testing algorithm.

APHL's HIV/Hepatitis Subcommittee developed this document through consensus to provide suggested reporting language and interpretation of test results for the HIV Laboratory Diagnostic Testing Algorithm. Members of this subcommittee include subject matter experts from state and local public health laboratories. The reporting language presented here is suggested and may need to be adapted to meet individual facility or jurisdiction requirements and modified over time as additional practical experience is gained with the revised algorithm.

The HIV Laboratory Diagnostic Testing Algorithm offers several advantages over the conventional algorithm of HIV antibody screening followed by Western blot confirmation of repeatedly reactive results, including earlier detection of HIV infections and the ability to accurately classify HIV-1 and HIV-2 infections. For maximum benefit of the algorithm, delayed testing should be avoided. Batching of specimens should be minimized, especially when supplemental testing is conducted.
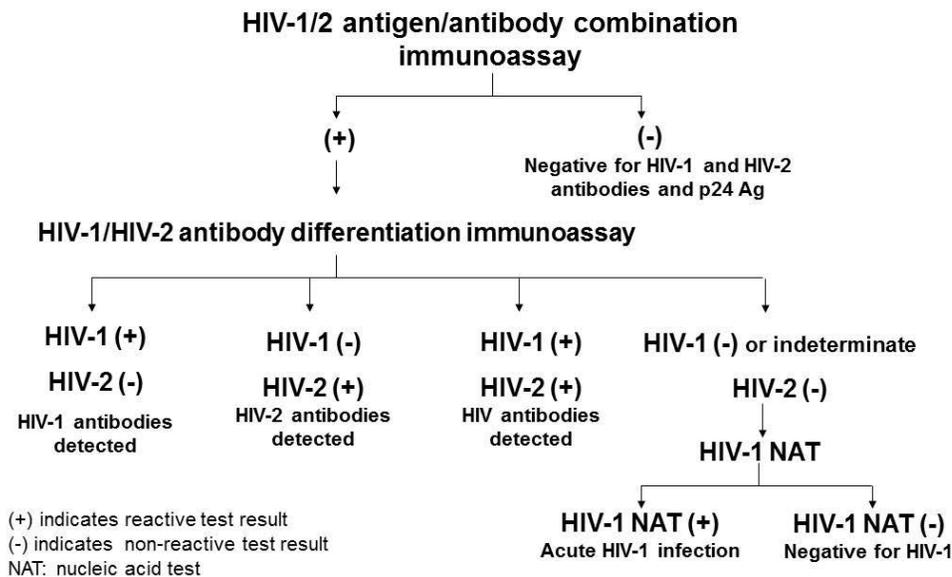
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1   Draft Laboratory Testing Guidance. Centers for Disease Control and Prevention; http://www.cdc.gov/hiv/testing/lab/guidelines/index.html. Accessed September 23, 2013

2   CLSI. Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline. CLSI Document M-53A, 2011
*CLSI Document M-53A is available only through purchase from CLSI.

3   DRAFT Recommendations: Diagnostic Laboratory Testing for HIV Infection in the United States http://www.cdc.gov/hiv/pdf/policies_Draft_HIV_Testing_Alg_Rec_508.2.pdf. Accessed September 23, 2013

Figure 1: Recommended HIV Laboratory Diagnostic Testing Algorithm for Serum or Plasma Specimens

HIV-1/2 antigen/antibody combination immunoassay

(+)

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

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)
HIV-2 (-)
HIV-1 antibodies detected

HIV-1 (-)
HIV-2 (+)
HIV-2 antibodies detected

HIV-1 (+)
HIV-2 (+)
HIV antibodies detected

HIV-1 (-) or indeterminate
HIV-2 (-)
HIV-1 NAT

HIV-1 NAT (+)
Acute HIV-1 infection

HIV-1 NAT (-)
Negative for HIV-1

(+) indicates reactive test result
(-) indicates non-reactive test result
NAT: nucleic acid test

Description of HIV Laboratory Diagnostic Testing Algorithm, Test Methods and Suggested Interpretation

The following description and table intends to clarify the test methods that are suitable for the algorithm above and guide how to report test results to persons providing testing for serum or plasma specimens. The HIV Laboratory Diagnostic Testing Algorithm is a sequence of multiple tests in which the final diagnostic interpretation relies on results from one or more tests. The tests outlined in this table are not suitable for oral fluid or dried blood spots.

Step 1: 4th Generation HIV-1/2 Antigen/Antibody Immunoassay

If the result of this test is "nonreactive," the interpretation is HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected; there is no laboratory evidence of HIV infection (Table 1). No further testing of the specimen is indicated. If the result is "reactive," this indicates possible presence of HIV-1 antibodies, HIV-2 antibodies and/or HIV-1 p24 antigen. This is considered a presumptive positive result and additional supplemental testing is required.

It is strongly recommended that laboratories use a 4th generation IA as the initial test; however, some laboratories may elect to use 3rd generation IAs. Although less sensitive than 4th generation IAs, 3rd generation IAs will detect some HIV-1 infections before the Western blot becomes positive. If a 3rd generation IA is used in step 1, the remainder of the algorithm should be followed because the HIV-1/HIV-2 antibody differentiation assay and HIV-1 NAT assay, in combination, provide fewer false negative results than the Western blot for specimens collected early after infection. If laboratories using 3rd generation IAs continue to perform Western blot testing for confirmation, specimens with a negative or indeterminate Western blot result should reflex to an HIV-1 NAT as outlined in step 3 of the algorithm. Reports should include a limitation statement noting the decreased sensitivity of

3rd generation IAs during the early phase of infection.

The HIV Laboratory Diagnostic Testing Algorithm should also be used for confirmation of all preliminary positive rapid HIV test results. Confirmatory testing of the serum or plasma specimen must start from step 1 of the algorithm.

Step 2: HIV-1/HIV-2 Antibody Differentiation Immunoassay

If the initial HIV-1/2 immunoassay (step 1) is 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; laboratory evidence of HIV-1 infection is present" or "positive for HIV-2 antibodies; laboratory evidence of HIV-2 infection is present" respectively. No further testing of the specimen is required and clinical follow-up 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; unable to differentiate between HIV-1 and HIV-2 antibodies." Reactive results are consistent with laboratory evidence of HIV infection and clinical follow-up is recommended. The clinician may consider additional testing for HIV-1 RNA and HIV-2 RNA or DNA to verify or rule-out the presence of HIV-1, HIV-2, or 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 NAT (step 3).

Step 3: HIV-1 NAT

If the initial HIV-1/2 immunoassay (step 1) is reactive and HIV-1 RNA is detected, the final interpretation is "positive for HIV-1; laboratory evidence of HIV-1 infection consistent with an acute or early HIV-1 infection." Clinical follow-up is recommended.

If HIV-1 RNA is not detected, the final interpretation is "HIV-1 antibodies were not confirmed and HIV-1 RNA is not detected; no laboratory evidence of HIV infection." The initial HIV-1/2 immunoassay result is a possible false positive. If there is reason to suspect recent HIV-2 infection, or if other clinical symptoms suggest immunosuppression, follow-up testing should be considered. Currently, there are no FDA approved HIV-2 RNA or DNA detection assays and those that are performed are laboratory developed tests.

In cases where the HIV-1/HIV-2 antibody differentiation assay (step 2) is "nonreactive" or "indeterminate" and the HIV-1 NAT assay (step 3) is either invalid or could not be performed, the algorithm is incomplete and cannot be fully interpreted. In this situation, the interpretation should be reported as "inconclusive." Whether the report is written or verbal will be situation dependent. An additional specimen should be requested and the entire algorithm should be repeated.

Table 1: Suggested Guidance for Reporting Results from the HIV Laboratory Diagnostic Testing Algorithm

	Test Sequence			Interpretation for Laboratory Report	Further Action**
	Step 1	Step 2	Step 3		
	Final Result from HIV-1/HIV-2 Ag/Ab IA*	HIV-1/HIV-2 Antibody Differentiation IA	HIV-1 NAT		
Test Outcomes	Nonreactive	n/a	n/a	HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection.	Sample can be reported as nonreactive for HIV. If recent HIV exposure is suspected, redraw and repeat algorithm.
	Reactive	Reactive for HIV-1	n/a	Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present.	Provide person tested with appropriate counseling and link to medical care
	Reactive	Reactive for HIV-2	n/a	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	Provide person tested with appropriate counseling and link to medical care
	Reactive	Undifferentiated: reactive for HIV-1 and HIV-2	n/a	Positive for HIV antibodies. Unable to differentiate between HIV-1 and HIV-2 antibodies.	Provide person tested with appropriate counseling and link to medical care and treatment. Clinician may consider additional testing for HIV-1 RNA and HIV-2 RNA or DNA to verify or rule out HIV-1/HIV-2 dual infection. Request additional specimen if original specimen volume is insufficient.
	Reactive	Nonreactive or indeterminate	Detected	Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an acute or early HIV-1 infection.	Provide person tested with appropriate counseling and link to medical care and treatment.
	Reactive	Nonreactive or indeterminate	Not detected	HIV-1 antibodies were not confirmed and HIV-1 RNA is not detected. No laboratory evidence of HIV infection. Possible false positive.	Consider repeat testing if clinically indicated. If there is a reason to suspect recent HIV-2 infection, additional testing for HIV-2 RNA or DNA should be considered.
	Reactive	Nonreactive or indeterminate	Invalid or not performed	Inconclusive	Request an additional specimen and repeat the algorithm.

* Repeating screening IA or initial reactive test is assay dependent
** Comments under "Further Action" can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with healthcare providers

Suggested language for reporting HIV test results is also available from CLSI[1] and the New York State Clinical Laboratory Evaluation Program.[4]

4   Shulman SH, Parker MM. Interim Guidelines for Laboratories on the use of a new Diagnostic Testing Algorithm for Human Immunodeficiency Virus (HIV) Infection 2013; https://www.health.ny.gov/diseases/aids/regulations/testing/docs/guidelines_diagnostic_testing.pdf. Accessed July 20, 2013.

Guidance on Reporting Test Results to Health Care Providers

The final interpretation of The HIV Laboratory Diagnostic Testing Algorithm is generated from a combination of HIV test results that health care providers may be unaccustomed to receiving. 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 beyond those tests that are routinely recommended on HIV positive patients (e.g. viral load CD4, and ARV resistance genotyping). Further actions are included in the table above to help guide submitters on appropriate next steps following testing.

The algorithm is intended for identifying new, previously undiagnosed HIV infections. Laboratories may receive specimens from previously diagnosed individuals, including individuals on antiretroviral treatment, for purposes of verifying infection status for the medical record. False negative test results could be produced under these conditions. Including a statement on the laboratory report indicating that the test results should be interpreted in the context of all clinically relevant information is recommended.

The following are some general guidelines to follow when reporting HIV test results to health care providers:

1. Laboratories should specify which assays were used (e.g., HIV-1/HIV-2 antigen/ antibody combination assay; HIV-1/HIV-2 antibody assay; HIV-1/HV-2 antibody differentiation assay, or other HIV-1 supplemental test) and the results of each assay.
2. If laboratories use a testing sequence that substitutes assays for those recommended in the algorithm, reports should describe the limitations associated with the testing sequence used.
3. In situations where tested persons might benefit, laboratories can report the result of each test in the algorithm as it becomes available without waiting for the final algorithm interpretation.
4. If the entire recommended testing algorithm is not completed, laboratories should specify which test results are pending, any additional tests that are necessary to establish the laboratory diagnosis and request any additional specimens required.
5. The diagnosis of acute HIV infection has implications for increased risk of transmission to uninfected partners and potential public health interventions. Laboratories should have arrangements in place to expedite reporting of test results indicative of acute HIV infection to the health care provider and to the health department.

Guidance on Laboratory Reporting for Surveillance

All states, the District of Columbia, and United States territories and dependent areas require that laboratories report test results indicative of HIV infection to the surveillance program in the department of health in the patient's jurisdiction of residence.[5] Requirements of state or local health departments might differ; therefore, follow the requirements of your jurisdiction. The following reporting principles will facilitate accurate case reporting related to the HIV Laboratory Diagnostic Testing Algorithm:

1. Results from the recommended laboratory testing algorithm with a negative overall interpretation (i.e., indicating that the patient is uninfected) should not be reported.
2. If the interpretation of the laboratory diagnostic testing algorithm is positive, indicating the presence of HIV infection, laboratories should report to the health department:
    a. the overall result or conclusion of the algorithm, **and**
    b. results from **all** tests (including negative/nonreactive or indeterminate results) performed as part of the testing algorithm (e.g., initial 4th or 3rd generation test result, HIV-1/2 differentiation test, and NAT) using the corresponding LOINC (Logical Observation Identifiers and Codes).
3. If the recommended laboratory diagnostic testing algorithm was not completed and the overall interpretation was inconclusive (indicating possible HIV infection that requires additional testing for confirmation), the laboratory should follow local requirements for reporting inconclusive results.

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5   CDC. *HIV/AIDS Surveillance Report, 2009.* Atlanta, GA: CDC;2011.

Acknowledgements

This document was developed by APHL's HIV/Viral Hepatitis Subcommittee with significant input from the following individuals:

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