LabWire
Laboratory News and Analysis for Clinicians November 2015

HIV Testing Changes: New 4th Generation Screen and Confirmation Testing

On November 2, 2015, Bronson Laboratory will change to new procedures for HIV screening and confirmatory testing. These changes reflect the most recent recommendations from the Centers for Disease Control and Prevention (CDC).[1] The CDC issued the updated recommendations for the following reasons:

- FDA approval of improved HIV assays that allow detection of HIV sooner after infection than previous immunoassays.
- Evidence that relying on Western blot or indirect immunofluorescence assay (IFA) for confirmation of reactive initial immunoassay results can produce false-negative or indeterminate results early in the course of HIV infection.
- Recognition that risk of HIV transmission from persons with acute and early infection is much higher than that from persons with established infection.
- Recent indications for the clinical benefits from antiretroviral treatment (ART) of all persons with HIV infection, including those with acute infection.
- Demonstration that the majority of HIV-2 infections detected by available HIV antibody immunoassays are misclassified as HIV-1 by the HIV-1 Western blot.

The improved HIV assays noted above are termed "4th generation" tests. These detect antibodies to HIV-1 and HIV-2 and the HIV-1 p24 antigen. Only antibodies are detected in 3rd generation tests. The pattern of emergence of these laboratory markers is consistent and allows classification of HIV infection into distinct laboratory stages described below (*see Figure 1*):

- **Eclipse Period** – The initial interval after infection with HIV when no laboratory markers are consistently detectable. This period ends approximately 10 days after infection when HIV-1 RNA becomes detectable by nucleic acid testing (NAT). Four to 10 days after the initial detection of HIV-1 RNA, HIV-1 p24 antigen is expressed and quantities rise to levels that can be detected by 4th generation immunoassays.
- **Acute HIV Infection** – The interval between the appearance of detectable HIV RNA and the first detection of antibodies. Its duration depends on the design of the antibody immunoassay and the sensitivity of the immunoassay during seroconversion.
- **Seroconversion Window Period** – The interval between infection with HIV and the first detection of antibodies. Immunoglobulin (IgM) antibodies can be detected by 3rd and 4th generation immunoassays approximately one week after p24 antigen is first detectable. The p24 antigen detection (*continued*)

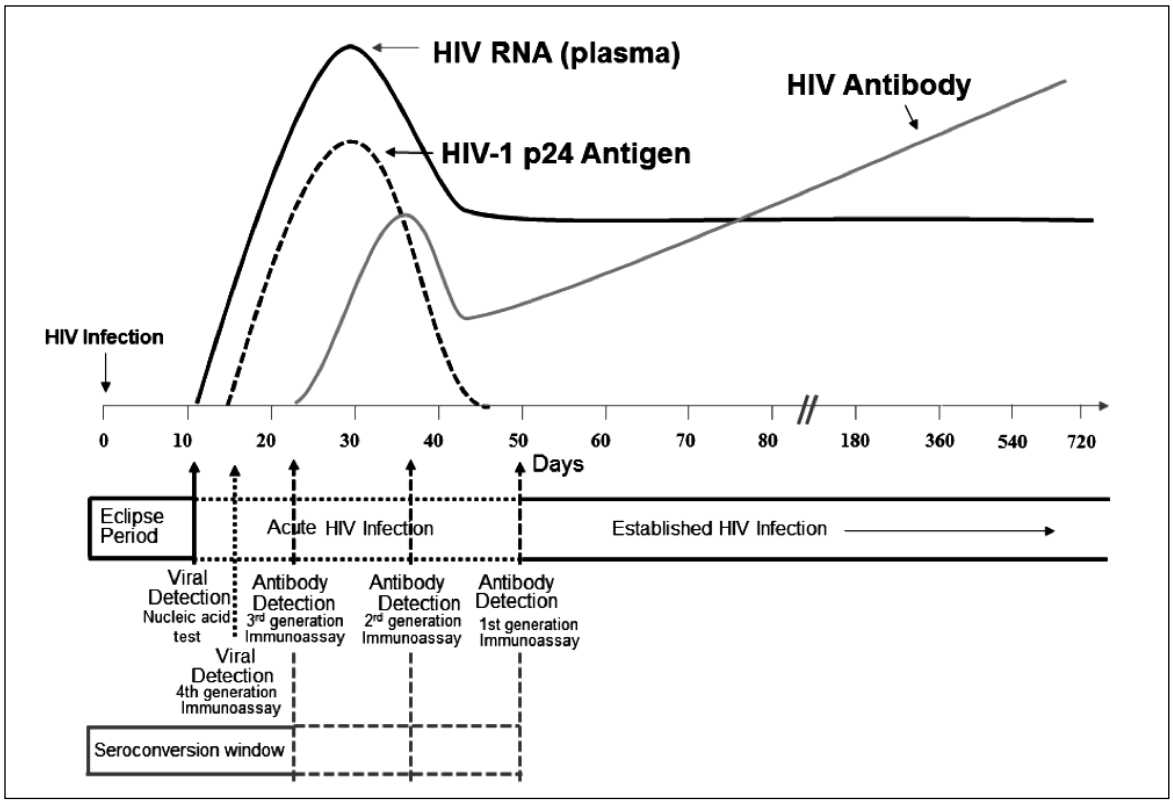


Figure 1. Sequence of appearance of laboratory markers for HIV-1 infection[1]

BRONSON

HIV Testing Changes: New 4th Generation Screen and Confirmation Testing

is transient because, as antibodies begin to develop, they bind to the p24 antigen and form immune complexes that interfere with p24 assay detection.

- **Established HIV Infection** – The stage characterized by a fully developed IgG antibody response.

The new recommended algorithm (*see Figure 2*) and specifics on the methods utilized at Bronson are described below:

- Laboratories should 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 to screen. No further testing is required for specimens that are nonreactive on the initial immunoassay.

  At Bronson, the initial testing is performed on the Siemens Advia Centaur with the 4th generation HIV 1/2 Combo Assay. This method only provides a positive or negative result; i.e. for positive results, it does not differentiate between antigen or antibody positivity.

- Specimens with a reactive antigen/antibody combination immunoassay result should be tested with an FDA-approved antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies.

  At Bronson, the Bio-Rad Geenius HIV 1/2 Supplemental Assay is the differentiation test. This test will be performed in-house daily and will provide test results three to five days quicker than the Western Blot assay formerly sent to a reference lab.

- 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).

  At Bronson, this test will be HIV-1 RNA by PCR which is sent to Mayo Medical Laboratories. A different specimen and a new order are required for that testing. The laboratory report will indicate this, and the laboratory will contact the provider to inform them.

Based upon the results of the HIV-1 NAT, the following interpretations may be made:

- A reactive HIV-1 NAT result and nonreactive HIV-1/HIV-2 antibody differentiation immunoassay result indicates laboratory evidence for acute HIV-1 infection.
- 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.
- 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.

The FDA algorithm also includes the comment, "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."

At Bronson, rapid HIV testing is performed as a STAT request for OB-Delivery patients and *(continued)*

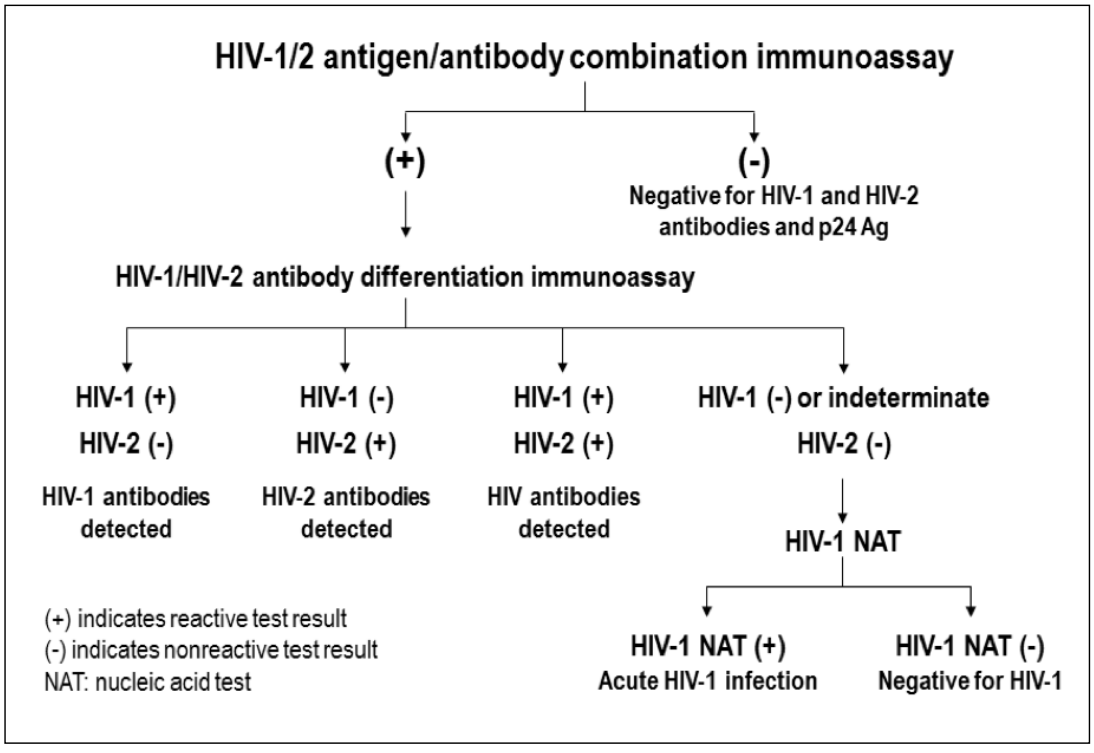


Figure 2. CDC Recommended Laboratory HIV Testing Algorithm

for STAT testing related to blood and body fluid exposures. For any laboratory screening test, there is a balance between optimal Sensitivity (i.e., a low number of false negatives) and optimal Specificity (i.e., a low number of false positives). For HIV testing, false negatives are to be avoided and so the trade-off for increased Sensitivity is decreased Specificity. False positives have been reported at Bronson from the rapid HIV test in use here (Alere Determine HIV Ag/Ab). With the implementation of the new algorithm on November 2, STAT requests may be tested directly by the new 4th generation assay. If the rapid test is needed to meet turnaround-time requirements, any positive rapid test will be retested the same shift by the new 4th generation assay. This should resolve many of the concerns raised by false positive rapid tests. Nonetheless, false positives are also a possibility with the new 4th generation assay. For this reason, the screening tests are reported as Preliminary Positive as shown below:

PRELIMINARY POSITIVE – Screening test for HIV is positive. Screening test is performed with the Advia Centaur HIV Ag/Ab Combo 4th generation immunoassay for the qualitative determination of p24 antigen and antibodies to the Human Immunodeficiency Virus types 1, 2 and group O. This test does not differentiate between p24 antigen and HIV antibody.

This specimen will be tested for HIV 1/2 antibody differentiation by immunoassay per CDC recommendations. Should further antigen specific testing be indicated, a new order and specimen will be required. The provider will be notified in such cases.

Medical evaluation and counseling is an important part of HIV testing and should include test result confirmation for any patient with initial positive results.

If you have questions about the new testing algorithm, please contact any of the following individuals:

Jeffrey Pearson, MD, Medical Director
(269) 341-8997

Ryan Johnson, MD, Pathologist
(269) 341-8997

Paul Guthrie, MLS, Core Lab Manager
(269) 341-7194

Paul Guthrie, MT ASCP

References

1. Centers for Disease Control and Prevention (CDC), Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations, Published June 27, 2014

http://www.cdc.gov/hiv/pdf/hivtestingalgorithmrecommendation-final.pdf

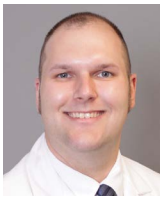
CoaguChek XS Point-of-Care Test Update

Recently, the maker of the CoaguChek XS PT test strips (Roche Diagnostics) determined that the test strips are affected by the presence of both unfractionated and low molecular weight heparins when the INR value is greater than 2.9. Specifically, when INR values are >2.9, the heparin neutralization is not sufficient to neutralize unfractionated heparin concentrations up to 0.8 U/mL and low molecular weight heparins (LMWH) up to 2 IU/mL. This will lead to falsely elevated point-of-care test results. Below an INR value of 2.9, the CoaguChek XS PT test strips allow for adequate neutralization of unfractionated heparin and LMWH.

If a patient receives unfractionated heparin or LMWH plus a vitamin K antagonist and the INR is greater than 2.9, the INR may be falsely elevated. In order to accurately assess the PT/INR in these situations, it is recommended that these tests be performed at Bronson Laboratory for confirmation.

Summary

- If a patient is receiving LMWH and warfarin, the INR should be checked just prior to the next scheduled LMWH dose.
- If a patient is on LMWH and warfarin with an INR at or above 2.9, a patient sample must be drawn and sent to the core lab for confirmation.
- The CoaguCheck XS system should not be used for patients being treated with any direct thrombin inhibitors, including Hirudin, Lepirudin, Bivalirudin and Argotroban.



Ryan Johnson, MD

LabWire is published by Bronson Laboratory Services. If you have a topic you would like addressed in this publication, call (269) 341-8997 or send your request to Jeff Pearson, MD, at pearsonj@bronsonhg.org.