UPDATE

Human Immunodeficiency Virus (HIV) 1/2 Antigen and Antibodies, Fourth Generation, with Reflexes

SUMMARY
Effective July 29, 2015, PeaceHealth Laboratories will implement changes in testing for the Human Immunodeficiency Virus (HIV) based on recommendations by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL). In accordance with these recommendations, a testing sequence of assays with serum or plasma specimens will be used to accurately diagnose HIV infection.

1. Routine HIV antibody testing will be performed using the 4th generation Bio-Rad HIV 1/2 Ag/Ab Combo EIA test with reflex to the Bio-Rad Multispot HIV-1/HIV-2 test for differentiation. Additional Nucleic Acid Testing (NAT) will be performed, if indicated. Additional information on this methodology is included in this publication. Order test code: 42300 HIV Ag/Ab 4th Generation Reflex to Confirmation.

2. Stat testing for HIV will be performed using the Alere Determine HIV-1/2 Ag/Ab rapid assay. All reactive specimens (preliminary positive) using the Alere Determine HIV-1/2 Ag/Ab rapid assay will be retested using the 4th generation Bio-Rad HIV 1/2 Ag/Ab Combo EIA with reflex to the Bio-Rad Multispot HIV-1/HIV-2 test for differentiation per the CDC recommendation listed below. Order test code: 4265 HIV-1/2 Ag/Ab Rapid reflex to HIV 1/2 Ag/Ab (4th Generation), if reactive.

Note: The Rapid HIV-1/2 Ag/Ab test is intended to be used in cases when an employee, patient, visitor or first responder is inadvertently exposed to blood or body fluids from another person and in emergency department situations.

RECOMMENDED ALGORITHM FOR SEQUENCE OF TESTING
The following sequence of testing assays with serum or plasma specimens will be used to accurately diagnose HIV infection as recommended by the CDC and APHL (see figure 1 on page 3):

1. 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 nonreactive specimens on this initial immunoassay.

2. Specimens with a reactive antigen/antibody combination immunoassay result will 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 will be interpreted as positive for HIV-1 antibodies, HIV-2 antibodies, or HIV-1 and HIV-2 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 will be tested with an FDA-approved HIV-1 NAT.

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

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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.

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

5. HIV-1 Western Blot is no longer part of the recommended algorithm for HIV testing.

**DISCONTINUED TESTS**

The tests listed below will be discontinued effective August 1, 2015. Panels containing HIV antibody testing will be updated to the new tests automatically and no additional action is required when ordering.

- 42000 Human Immunodeficiency Virus (HIV) 1 and 2 Antibodies, Reflex by Western Blot
- 42002 Human Immunodeficiency Virus with Western Blot, if indicated
- 42003 Human Immunodeficiency, Prenatal
- 42004 Human Immunodeficiency Virus (HIV) Prenatal, Reflexed by Western Blot, if indicated
- 42156 Human Immunodeficiency Virus-1 (HIV-1) Antibody, Rapid; Reflex by Western Blot
- EXP_SOURCE Exposure, Source
- EXP_EXPOSED Exposure, Exposed
- HIV, HIV 1 and 2, Human Immune Deficiency Virus

**NEW RECOMMENDED ALGORITHM ADVANTAGES**

This recommended algorithm has several advantages over previous recommendations, including:

- more accurate laboratory diagnosis of acute HIV-1 infection, i.e. detection of HIV-1 p24 antigen
- equally accurate laboratory diagnosis of established HIV-1 infection
- more accurate laboratory diagnosis of HIV-2 infection
- fewer indeterminate results
- faster turnaround time for most test results

Advancements in HIV testing technology and early detection of HIV may maximize the benefits to individual and public health.

The detection of HIV-1 p24 antigens allows for the identification of acute HIV-1 infection. Compared with previous testing recommendations, the updated algorithm increases sensitivity for acute HIV-1 infection by including an initial immunoassay that detects antibodies to both HIV-1 and HIV-2 and also HIV-1 p24 antigen, which can be detected before antibodies develop.

The updated algorithm identifies acute HIV-1 infection by using HIV-1 NAT for specimens that are reactive on the initial immunoassay but negative for antibodies on the second immunoassay.
Figure 1: HIV Testing Algorithm for Serum or Plasma Specimens

- **HIV-1/2 Antigen/Antibody Combination Immunoassay**
  - Positive (+)
  - Negative (-) for HIV-1 and HIV-2 antibodies and p24 antigen

- **HIV-1 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 of HIV-1

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## ORDERING INFORMATION

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<tr>
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<td></td>
<td>4th Generation</td>
<td>Confirmation by Multispot</td>
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## SPECIMEN REQUIREMENTS

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<tr>
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<th>Standard Vol</th>
<th>Minimum Vol</th>
<th>Transport</th>
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<tr>
<td>Two 7.5 mL serum separator tubes (SST). Also acceptable: Two 5 mL red top tubes or, two lavender top tubes (EDTA).</td>
<td>Allow to clot, centrifuge and separate serum or plasma from cells immediately and pour into a plastic vial. Refrigerate. If specimen cannot be assayed within 7 days, freeze serum or plasma at -20 degrees C or colder. Specimen should not be used if frozen/thawed more than 4 times.</td>
<td>Ambient – 2 days Refrigerated – 7 days Frozen – 1 year</td>
<td>2.5 mL serum or plasma</td>
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<td>3.0 mL serum or plasma</td>
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## REFERENCES