Overview

• Current Recommendations
• Rationale for Testing Algorithm
• Applying Testing Algorithm and Recommendations to Your Program
National HIV/AIDS Strategy (NHAS)
Released July 2010

- **REDUCE NEW INFECTIONS**
  - Reduce new infections (25%)
  - Reduce transmission rate (30%)
  - Increase awareness of HIV+ serostatus to 90% PLH

- **INCREASE ACCESS TO CARE**
  - Link 85% of PLH to care within 3 mo of diagnosis
  - Increasing to 80% RW clients in continuous care
  - Increase to 86% RW clients in permanent housing

- **REDUCE HIV-RELATED HEALTH DISPARITIES**
  - Increase the share of HIV diagnosed gay/bisexual men, blacks and Latinos with an undetectable VL by 20%
NY’s Plan to End HIV Epidemic

- Identifying persons with HIV who remain undiagnosed and linking them to health care
- Linking and retaining persons diagnosed with HIV to health care and getting them on therapy to maximize viral suppression
- Facilitating access to Pre-Exposure Prophylaxis (PrEP) for high-risk persons to keep them HIV negative
HIV DIAGNOSTIC ALGORITHMS
• Public Health Service recommends that no positive test results be given to clients/patients until a screening test has been repeatedly reactive on same specimen and supplemental, more specific test such as the Western blot has been used to validate those results.
Western Blot Assay

• First supplemental test approved by FDA to confirm diagnosis of HIV-1 infection
• Method in which HIV-1 proteins are separated according to size by gel electrophoresis
• Proteins are transferred to paper and reacted to patient’s serum
• Any HIV IgG antibody to those proteins appears as band
1989: State of the Art

Enzyme Immunoassay (EIA)

Western blot
The first tests detect HIV IgG antibodies.

Antigen:
1st - Viral lysate
2nd - Recombinant proteins or synthetic peptides

Plasma/serum

IgG HIV antibody

Enzyme detection

Anti-human IgG

Dects HIV IgG

Color reagent

The first tests detect HIV IgG antibodies.
Later tests detect HIV IgG and IgM antibodies.
Screening HIV Tests

Traditional/Conventional Tests

• Enzyme Immunoassay (EIA)
  – HIV antibody screening test for blood or oral fluid specimens
  – Processed on analyzers in laboratory settings
  – Reactive results are preliminary & must be confirmed

Approximately 25 million persons each year in the United States are tested for antibody to human immunodeficiency virus (HIV). Publicly funded counseling and testing (CT) programs conduct approximately 2.5 million of these tests each year. CT can have important prevention benefits (1); however, in 1995, 25% of persons testing HIV-positive and 33% of persons testing HIV-negative at publicly funded clinics did not return for their test results (2). Rapid tests to detect HIV antibody can be performed in an average of 10 minutes (3), enabling health-care providers to supply definitive
Notice to Readers

Protocols for Confirmation of Reactive Rapid HIV Tests

On November 7, 2002, the Food and Drug Administration (FDA) announced approval of the OraQuick® Rapid HIV-1 Antibody Test (OraSure Technologies, Inc., Bethlehem, Pennsylvania) for use by trained personnel as a point-of-care test to aid in the diagnosis of infection with human immunodeficiency virus type 1 (HIV-1). Subsequently, two other rapid HIV tests have been approved by FDA: the Reveal™ HIV-1 Antibody Test (MedMira Laboratories, Halifax, Nova Scotia) and the Uni-Gold Recombigen™ HIV Test (Trinity Biotech, Wicklow, Ireland).

All reactive rapid HIV test results require confirmatory testing. CDC described protocols for confirming reactive rapid HIV tests based on a consultation convened in January 2003 with expert laboratory scientists, FDA, and the Centers for Medicare and Medicaid Services (1). These protocols recommend 1) confirmation of all reactive rapid HIV test results with either Western blot (WB) or immunofluorescent assay (IFA), even if an enzyme immunoassay (EIA) screening test is negative, and 2) follow-up testing for persons with negative or indeterminate confirmatory test results, with a blood
Rapid/Point-of-Care HIV Ab Tests
Laboratory Testing for the Diagnosis of HIV Infection

Updated Recommendations

Published June 27, 2014
First update since 2004
RATIONALE FOR NEW RECOMMENDATIONS
Rationale for New Recommendations

• Previous testing algorithm for HIV-1 fails to identify acute HIV-1 infections
  – HIV-1 Western blot and HIV-1 IFA no longer part of recommended algorithm
• Assays that detect HIV-1 infection earlier are now widely available
Acute HIV Infection (AHI)

Diagnostic Challenge

- Clinicians need to be alert to diagnose AHI
  - Approximately 50% individuals with AHI are asymptomatic (but are also highly infectious)
  - Nonspecific symptoms and physical findings, similar to flu or mononucleosis
    - Fever, rash, sore throat, diarrhea, muscle aches, swollen glands

- Routine HIV antibody screening tests cannot diagnose AHI
p24 antigen: viral core protein that transiently appears in blood during ramp up period after HIV RNA appears
Western Blot is Technology Much Older Than Screening Tests
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How Well Do Current Technologies Confirm Diagnoses?
Sequence of Test Positivity Relative to WB
Many HIV Tests Can Detect HIV Infections Before WB Does

166 plasma specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency

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Risk of Sexual Transmission of HIV

Risk of Transmission Reflects Genital Viral Burden

HIV RNA in Semen (Log_{10} copies/ml)

1/30 - 1/200

1/1000 - 1/10,000

1/500 - 1/2000

1/100 - 1/1000

Acute Infection
3 wks

Asymptomatic Infection

HIV Progression

AIDS

Rationale for New Recommendations

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• Assays that detect HIV-1 infection earlier are now widely available
• Risk of HIV-1 transmission from persons with acute and early infection is much higher than that from persons with established infection
  • Up to 50% of new HIV infections were transmitted during the acute phase\(^1,2\)

\(^1\)Pinkerton S, *AIDS Behavior*, 2008
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• Initiation of ART during early state of HIV-1 infection can benefit patients & reduce HIV transmission
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• Initiation of ART during early state of HIV-1 infection can benefit patients & reduce HIV transmission
• Use of HIV-1 Western Blot in previous algorithm misclassifies majority of HIV-2 infections
HIV Type 2 in NYC, 2000–2008

• 62 confirmed or probable HIV-2 infections
  – Majority (97%) were foreign-born
  – 17.7% had AIDS at initial diagnosis of HIV-2
  – 40 (64.5%) were initially diagnosed with HIV-1
    • HIV-2 virus 40-60% similar to HIV-1
    • HIV-2 antibodies can cross-react with WB test and provide false positive or false indeterminate result for HIV-1

Torian, CID. 2010;51:1334-1342.
DOHMH Recommendation to Testing Contractors

January 7, 2015

Dear Colleagues,

We wish to inform you of a new development in HIV testing technology with important implications for testing programs.

- Recently, the US Food and Drug Administration (FDA) granted CLIA waivers to two additional point-of-care HIV tests, including the Alere Determine™ HIV-1/2 Antigen/Antibody (Ag/Ab) Combo Test, the first fourth-generation point-of-care test. The CLIA waiver of this test facilitates the diagnosis of acute and early HIV infection in many clinical and non-clinical settings.
- Because the risk of HIV transmission from persons during acute and early HIV infection is much greater than that from persons with established HIV infections, testing programs should:
  o Attempt to integrate into their programs the use of testing technology that allows for earlier detection of HIV infection, such as the HIV Ag/Ab combo test.
  o Follow CDC’s recommended HIV testing algorithm for confirming reactive results from screening tests, instead of using the Western blot for confirmation.

Background

Recently, the FDA has granted CLIA waivers to two additional point-of-care HIV tests, the Alere Determine™ HIV-1/2 Ag/Ab Combo Test and the ChemBio Dual Path Platform (DPP)™ HIV 1/2 Assay. There are now seven CLIA-waived point-of-care HIV tests approved for use in the United States. The FDA’s approval of improved HIV assays allows for detection of HIV earlier after infection than previous HIV assays. The risk of HIV transmission from persons with acute and early infection is much greater than that from persons with established infection. In some studies, nearly 50% of new infections could be attributable to transmission during acute stages of infection. Detection of acute and early HIV infection allows persons with HIV to be rapidly linked to medical care, to benefit from treatment, and to reduce the risk of HIV transmission to their partners.

In light of this development, the New York City Health Department now recommends that programs implement a testing technology that allows for earlier detection of HIV infection, such as the Alere Determine™ HIV-1/2 Ag/Ab Combo Test (fourth-generation test) and the bioLytical INSTICT™ HIV-1 Antibody Test. As before, for testing in laboratory settings, NYC DOHMH’s and CDC’s recommended screening HIV test is the HIV-1/2 antigen/antibody combination (fourth-generation) immunoassay.
DOHMH Recommendations

• Testing programs should implement testing technology that allows for earlier detection of HIV infections
  – Laboratory based EIA
    • Abbott Architect
    • Bio-Rad GS
    • Siemens ADVIA Centaur
    • Bio-Rad BioPlex 2200
  – Rapid, Point-of-Care
    • Alere Determine
HIV Testing Algorithm
Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens

1. 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 test for established HIV-1 or HIV-2 infection 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 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.

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.
Key Points

• Conduct screening with FDA-approved antigen/antibody combination immunoassay
  – Insufficient data to recommend use of rapid combination test (Determine®) in algorithm

• Specimens with reactive result should be tested with antibody immunoassay that differentiates HIV-1 and HIV-2 antibodies
  – Reflexes to HIV-1 nucleic acid test if test result from differentiation assay is non-reactive or indeterminate

• Laboratories reactive result on rapid HIV test, use testing algorithm starting with antigen/antibody combination immunoassay
Recommended HIV Testing Algorithm

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 nonreactive test result

NAT: nucleic acid test
APPLYING ALGORITHM & RECOMMENDATIONS
Applying Algorithm & Recommendations

**Choosing a Test**

- Choose HIV test that both allows for early detection of HIV infection and best fits testing program
  - If phlebotomy is being performed, use lab-based combo Ag-Ab test
Laboratory Combo Ag-Ab HIV Tests

- Enzyme Immunoassay
  - Abbott ARCHITECT
  - Bio-Rad GS
  - Siemens ADVIA Centaur
  - Bio-Rad BioPlex 2200
LabCorp: HIV Ag/Ab Combo with Reflex

Test Number: 083935

“with Reflex” means that specimens that produces reactive results on 4th generation test would have the cascade of tests recommended by the algorithm automatically ordered:

- HIV-1/HIV-2 antibody differentiation test, if 4th gen reactive
- HIV-1 viral load, if differentiation test negative or indeterminate
Quest: HIV Ag/Ab Combo with Reflex

Test Code: 91431

Test Name
HIV-1/2 Antigen and Antibodies, Fourth Generation, with Reflexes

CPT Code(s)
83739

Includes
If HIV Antibody and Antibody, 4th Generation Screen is Repeatedly Reactive, HIV-1/2 Antibody Differentiation will be performed at an additional charge (CPT code(s): 88701, 88702).

If HIV-1/2 Antibody Differentiation is Indeterminate or Negative, HIV-1 RNA, Qualitative, TMA will be performed at an additional charge (CPT code(s): 87535).

91432 HIV-1/2 Antibody Differentiation
BioReference: HIV Ag/Ab (B688-3)

Testing per algorithm is NOT reflexed

ORDERING
Test Code: B688-3
Turnaround Time: 1 days
Preferred Specimen: 1 mL SST Tube
Alternative Specimen:
Red Top, Aliquot Tube-Serum, Microtainer - Pediatric Red, Microtainer - Pediatric SST
Specimen Comment:
Positive result will auto-reflex to F171 HIV 1/2 DIFFERENTIATION at additional charge
Storage Instruction: Refrigerate

BILLING
CPT Codes: 87389 (1)
Applying Algorithm & Recommendations

Choosing a Test

• Choose HIV test that both allows for early detection of HIV infection and best fits testing program
  – If phlebotomy is being performed, use lab-based combo Ag-Ab test
  – If phlebotomy is not possible and/or rapid result is necessary, use point-of-care combo Ag-Ab test

• Other consideration:
  – Patient Flow & Processing time
    » 20 minutes for Determine, 1 minute for INSTI
  – Patient acceptability of testing
    » Fingerstick vs. Oral Fluid
    » Processing time
Recommended Rapid HIV Tests

- **Alere Determine**
  - Combo Ag-Ab (4\textsuperscript{th} g.)
  - 50 uL blood
  - 20 min to process

- **bioLytical INSTI**
  - Ab only
  - 50 uL blood
  - 1 min to process
Applying Algorithm & Recommendations

*Communicating about Window Period*

- **Window period depends on:**
  - Ability of test to detect an infection
  - Individual response to infection
    - Incubation period varies by people
    - Some people produce virus and antibodies faster than others
      - Some people can be detected earlier than others with same time of exposure
- **Communicating window period means**
  - When is the earliest that I can make a diagnosis?
  - How long do I have to wait before I can tell someone that s/he does not have an infection?
Applying Algorithm & Recommendations

Communicating about Window Period

- **Lab-based combo Ag-Ab test:**
  - Detects HIV infections **as early as** 14–15 days after potential exposure
  - Detects **most infections** by 28 days after exposure

- **Point-of-Care combo Ag-Ab test (Determine):**
  - On plasma specimens, Determine detected HIV infection **3–4 days after** lab-based 4th gen test
    - 1–3 days before lab-based 3rd gen test
    - 1–2 weeks before other rapid tests
  - Limited data on relative sensitivity of Determine combo and other rapid HIV tests when used with whole blood specimens
CDC Evaluation of Determine™

Performance on Plasma Samples

• Determine™
  – Detected fewer HIV infection than Architect, 88 (Determine) vs. 107 (Architect), (p<0.0001)
  – Detected 52.6% acute HIV infections, 20 (Determine) vs. 38 (Architect), (p<0.0001)

Masciotra S. Evaluation of Determine. CROI 2015, abstract #620
CDC Evaluation of Determine™
Performance on Simulated Whole Blood

• Created whole blood samples by adding RBCs to plasma samples
• Determine™ performance compared between whole blood and matched plasma samples
  – Fewer infections detected from whole blood than plasma, 36 (whole blood) vs. 52 (plasma)
  – Reactive result delayed in whole blood
    • Median delay of 2 days, overall
    • 7/12 samples showed delayed response (med. 5 days)
  – Determine whole blood detected more infections than OraQuick using whole blood or plasma

Masciotra S. Evaluation of Determine. CROI 2015, abstract #620
Applying Algorithm & Recommendations

Communicating about Window Period

• Recap
  – HIV viral load test
    • Detects HIV infections as early as 7 days after potential exposure
    • Detects most infections by 21 days after exposure
  – Lab-based combo Ag-Ab test
    • Detects HIV infections as early as 14–15 days
    • Detects most infections by 28 days after exposure
  – Point-of-Care combo Ag-Ab test (Determine)
    • Based on limited information
    • Detects HIV infections as early as 21 days
    • Detects most infections by 40 days after exposure
Applying Algorithm & Recommendations

Suspecting Acute Infection in Priority Group

• If client belongs to group at epidemiologic high risk for HIV, such as men who have sex with men or transgender women, **AND**

• 4\textsuperscript{th} generation test is negative, **AND**

• Exposure in past 28 days is suspected
  – Further testing should be considered, especially if client has symptoms suggestive of AHI
    • Viral load testing (detect HIV RNA as early as 7 days post-infection, with most detected by 21 days)
    • Repeat 4\textsuperscript{th} gen test if two weeks have transpired since first specimen was drawn
Applying Algorithm & Recommendations

Confirmatory/Supplemental Testing

• Per CDC recommendations, when a lab-based combo Ag-Ab test produces reactive result,
  – Use antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies, such as:
    • Bio-Rad Multispot HIV-1/HIV-2 Rapid Test
    • Bio-Rad Geenius™ HIV-1/HIV-2 Supplemental Assay
  – If supplemental assay is negative or indeterminate, HIV-1 viral load should be conducted as an acute infection is a possibility

• All persons with HIV should be referred to medical care and partner services
Choice of Supplemental Test

*In-the-Field Testing*

- If using Determine or INSTI in the field, following a reactive result, choices for supplemental test include:
  - Draw a venous blood sample to process according to testing algorithm (4th gen. test, followed by MultiSpot)
  - Use current supplemental test (oral fluid Western blot or dried blood spot specimen)
    - Most will be established infections so current test is fine
    - If supplemental test result is negative, contact patient to get specimen for NAAT/PCR testing to rule out AHI
  - Refer to clinical facility for supplemental testing
Alere Determine Test Results
Alere Determine Test Results

Acute infection
Sequence of Appearance of Lab Biomarkers for HIV

- **HIV RNA (plasma)**
- **HIV Antibody**
- **HIV p24 Ag**

Exposure to HIV:
- 1st gen HIV test
- 2nd gen
- 3rd gen

Time from exposure:
- 0
- 10
- 20
- 30
- 40
- 50
- 60
- 70
- 80
- 90
- 100
Alere Determine Test Results

Acute infection

Undergoing seroconversion
Sequence of Appearance of Lab Biomarkers for HIV

Exposure to HIV

Time from exposure

- HIV RNA (plasma)
- HIV p24 Ag
- HIV Antibody

- 1st gen HIV test

- 2nd gen

- 3rd gen

- 1st gen HIV test
Alere Determine Test Results

Acute infection

Undergoing seroconversion

Established infection
Sequence of Appearance of Lab Biomarkers for HIV

- HIV RNA (plasma)
- HIV Antibody

Exposure to HIV

Time from exposure

11 16 22

1st gen HIV test

2nd gen

3rd gen
Alere Determine Test Results

Refer to clinical provider for viral load testing, unless phlebotomy is available

Continue use of current conf. test
Applying Algorithm & Recommendations

Other Considerations

• If HIV exposure occurred in 36 hours prior to presentation…
  – Consider emergency post-exposure prophylaxis

• If tested negative and individual at ongoing risk of HIV infection…
  – Consider referral for pre-exposure prophylaxis
Next Steps

- Notify CLEP program of changing technology
- Update QA protocols for new testing technology
- Train staff for new technology
Testing Technology Representatives

• Alere Determine
  – Kevin Williams
  – 917-533-0641
  – Kevin.M.Williams@alere.com

• bioLytical INSTI
  – Leahjane Lavin
  – 704-615-2058
  – llavin@biolytical.com
Thank You