FOURTH GENERATION HIV TESTING

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Introduction

- 2010: estimated 1.1 million persons in the US were living with human immunodeficiency virus (HIV) infection, of whom an estimated 181,000 were unaware of their infection.
- Approximately 49,000 new HIV diagnoses are reported to CDC each year.
- Estimated number of new infections has remained stable at approximately 50,000 annually from 2008 to 2010.
- As of 2009, an estimated 83 million adults aged 18-64 years reported they had been tested for HIV.
- Accurate laboratory diagnosis of HIV is essential to identify persons who could benefit from treatment, to reassure persons who are uninfected, and to reduce HIV transmission.

Scope

- The updated recommendations for HIV testing are intended only for testing of serum or plasma specimens from adults and children aged 2 years or older.
- These recommendations do not include ages less than 2 years because maternal antibodies against HIV might be present in uninfected infants born to HIV-infected mothers.
- These recommendations do not address methods or strategies for screening blood or organ donors for HIV infection.

Background and rationale

- Accurate laboratory diagnosis of HIV infection relies on testing algorithms that maximize overall sensitivity and specificity by employing a sequence of tests in combination and applying decision rules for resolving discordant test results.
- Since 1989, the diagnostic algorithm for HIV testing in the US recommended by the CDC and APHL initiated testing with a sensitive HIV-1 antibody immunoassay.
- Specimens with repeatedly reactive initial immunoassays were then tested with a more specific HIV-1 antibody test, either the HIV-1 Western blot or HIV-1 indirect immunofluorescence assay (IFA), to validate those results.

Background and rationale

- In 1992, CDC recommended specific testing for both HIV-1 and HIV-2 antibodies:
  - If demographic or behavioral information suggested that HIV-2 infection might be present;
  - If there was clinical evidence or suspicion of HIV disease in the absence of a positive test for antibodies to HIV-1;
  - In cases in which the HIV-1 Western blot exhibited an unusual indeterminate pattern.
- In 2004, CDC recommended confirmation of all reactive rapid HIV test results with either HIV-1 Western blot or HIV-1/IFA, irrespective of intermediate immunoassays that may have been conducted.
- Since those recommendations were issued, improved immunoassays, an HIV-NAT, and a differentiation immunoassay that distinguishes HIV-1 from HIV-2 antibodies received FDA approval for use in diagnosis of HIV infections. These developments prompted reevaluation of recommendations for HIV diagnostic testing.
Updated recommendations

- Previous guidelines for serodiagnosis of HIV Type 1 infections employed only tests for HIV antibodies.
- Updated recommendations also include tests for HIV antigens and HIV nucleic acid.
- Studies from population at high risk for HIV demonstrate that antibody testing alone might miss a considerable percentage of HIV infections detectable by virologic tests.

Updated recommendations

- CDC and Association of Public Health Laboratories (APHL) have issued new recommendations based on HIV tests approved by the Food and Drug Administration (FDA) as of December 2012 and scientific evidence, laboratory experience, and expert opinion collected from 2007 through December 2013.
- These recommendations do not include the rapid HIV-1/HIV-2 antigen/antibody combination test approved by the FDA in August 2013 (for which evidence of performance in the algorithm was insufficient) or HIV-2 nucleic acid tests (NATs), which lack FDA approval.

Updated recommendations for HIV testing

- These updated recommendations are necessary because of:
  - 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 immunoassays 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; and
  - 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.

HIV IMMUNOASSAYS

Evolution of HIV Immunoassay Technology

- HIV immunoassays are generally grouped into “generations”:
  - 1st generation:
    - All antigens used to bind HIV antibodies are from a lysate of HIV-1 viruses grown in cell culture.
    - An indirect immunoassay format employs labeled antihuman IgG for detection of IgG antibodies.
    - Significant specimen dilution is required to overcome cross-sensitivity with cellular protein contaminants.
    - Examples available in the US include the HIV-1 Western blot and the HIV-IFA.
  - 2nd generation:
    - Synthetic peptide or recombinant protein antigens alone or combined viral lysates are used to bind HIV antibodies. An indirect immunoassay format employs labeled antihuman IgG or protein A (which binds to IgG with high affinity) for detection of IgG antibodies.
    - Design of the specific antigenic epitopes improves sensitivity for HIV-1 group O and HIV-2.
    - Eliminating cellular antigens that contaminate viral lysates improves specificity by eliminating cross-reactivity with cellular proteins.
    - Examples available in the US include one HIV-1 enzyme immunoassay and six rapid HIV antibody tests.
Evolution of HIV Immunoassay Technology

• 3rd generation
  - Synthetic peptide or recombinant protein antigens are used to bind HIV antibodies in an immunometric antigen sandwich format (HIV antibodies in the specimen bind to HIV antigens on the assay substrate and to antigens conjugated to indicator molecules).
  - This allows detection of IgM and IgG antibodies. Lower sample dilutions and the ability to detect IgM antibodies (which are expressed before IgG antibodies) increase sensitivity during early seroconversion.
  - Examples available in the US include one HIV-1/HIV-2 enzyme immunoassay and two HIV-1/HIV-2 chemiluminescent immunoassays.

• 4th generation
  - Synthetic peptide or recombinant protein antigens are used in the same antigen sandwich format as 3rd generation to detect IgM and IgG antibodies.
  - Monoclonal antibodies are also included to detect p24 antigen.
  - Inclusion of p24 antigen capture allows detection of HIV-1 infection before seroconversion.
  - These assays (termed “combo” assays) usually do not distinguish antibody reactivity from antigen reactivity.
  - Examples available in the US include one HIV-1/HIV-2 enzyme immunoassay, one HIV-1/HIV-2 chemiluminescent immunoassay, and one HIV-1/HIV-2 rapid test that uses separate indicators for antigen and antibody reactivity.

FDA Approved Assays

<table>
<thead>
<tr>
<th>Assay name</th>
<th>Manufactured</th>
<th>Algorithm used to detect reactivity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1/HIV-2 enzyme immunoassay</td>
<td>Abbott Center for AIDS Research, Chicago, IL</td>
<td>Positive signal to background ratio</td>
<td>In light</td>
</tr>
<tr>
<td>HIV-1/HIV-2 chemiluminescent immunoassay</td>
<td>Abbott, Abbott Park, IL</td>
<td>Positive signal to background ratio</td>
<td>In light</td>
</tr>
<tr>
<td>HIV-1/HIV-2 rapid test</td>
<td>Abbott, Abbott Park, IL</td>
<td>Positive signal to background ratio</td>
<td>In light</td>
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LABORATORY MARKERS OF HIV INFECTION

• Immediately after HIV infection, low levels of HIV-1 RNA might be present intermittently, but no viral markers are consistently detectable in plasma.
• Approximately 10 days after infection, HIV-1 RNA becomes detectable by NAT in plasma and quantities increase to very high levels.
• Next, p24 antigen is expressed and quantities rise to levels that can be detected by 4th generation immunoassays within 4 to 10 days after the initial detection of HIV-1 RNA. However, p24 antigen detection is transient because, as antibodies begin to develop, they bind to the p24 antigen and form immune complexes that interfere with the p24 assay detection unless the assay includes steps to disrupt the antigen-antibody complexes.
Laboratory markers of HIV

- Next, the IgM antibodies are expressed which can be detected by 3rd and 4th generation immunoassays 3 to 5 days after p24 antigen is first detectable, 10 to 13 days after the appearance of viral RNA.
- Finally, IgG antibodies emerge and persist throughout the course of HIV infection. 1st and 2nd generation immunoassays designed to detect only IgG antibodies exhibit considerable variability in their sensitivity during early infection, becoming reactive 18 to 38 days or more after the initial detection of viral RNA.

Laboratory markers of HIV

- The pattern of emergence of laboratory markers is highly consistent and allows classification of HIV infection into distinct laboratory stages:
  - The eclipse period- initial interval after infection with HIV when no laboratory markers are consistently detectable.
  - The seroconversion- interval between infection with HIV and the first detection of antibodies. Its duration depends on the design of the antibody immunoassay and the sensitivity of the immunoassay during seroconversion.
  - Acute HIV infection- interval between the appearance of detectable HIV RNA and the first detection of antibodies. Its duration also depends on the design of the antibody immunoassay and the sensitivity of the immunoassay during seroconversion.
  - Established HIV infection- stage characterized by a fully developed IgG antibody response sufficient to meet the interpretive criteria for a positive Western blot of IFA.

Need for updated recommendations

- Assays that detect HIV-1 infection earlier are now widely available.
- New generations of immunoassays with improved sensitivity for detecting early HIV-1 infection can narrow the interval between the time of infection and initial immunoassay reactivity.
- In 2006, 74% of US public health laboratories used a 1st or 2nd generation immunoassay as the initial test in the previous algorithm.
- In 2012, 92% of public health laboratories used a 3rd or 4th generation immunoassay as the initial test in the previous algorithm. However, these immunoassays become reactive days to weeks before the HIV-1 Western blot becomes positive. Using the HIV-1 Western blot for confirmation of these immunoassays can produce false-negative results during seroconversion.

Need for updated recommendations

- The previous testing algorithm for HIV-1 fails to identify acute HIV-1 infections.
- Since 1999, blood screening centers in the US have used pooled HIV-1 NAT to identify acute HIV infection in donors who had non-reactive 3rd generation immunoassay results. (To reduce costs, multiple specimens are pooled for screening with a single NAT; specimens from reactive pools undergo individual NAT to identify the specimen with HIV-1 RNA).
- Retrospective testing of specimens from high-risk persons demonstrated that 3rd generation immunoassays were reactive in 20% to 37% of specimens that were HIV-1 Western blot negative but NAT-reactive, and that 4th generation immunoassays were reactive in 62% to 83% of specimens that were NAT-reactive but nonreactive with earlier generation immunoassays.

Need for updated recommendations

- The risk of HIV-1 transmission from persons with acute and early infection is much higher than from persons with established infection.
- Extremely high levels of infectious virus become detectable in serum and genital secretions during acute HIV-1 infection and persist for 10-12 weeks. Models based on data from cohort studies suggest that the rate of sexual transmission during acute infection is 26 times as high as that during established HIV-1 infection.
- Acute HIV-1 infection, despite its short duration, can account for 10-50% of all new HIV-1 transmissions, especially in persons with multiple concurrent sex partners or high rates of partner change.
Need for updated recommendations

- Initiation of antiretroviral therapy (ART) during the early stage of HIV-1 infection can benefit patients and reduce HIV transmission.
- Treatment of acute and early HIV-1 infection with combination ART improves laboratory markers of disease progression. Limited data also suggest that treatment of acute HIV-1 infection might decrease the severity of acute disease, lower the size of the viral reservoir, and decrease the rate of viral mutation by suppressing viral replication and preserving immune function.
- Because very high levels of virus in blood and genital secretions increase infectiousness during and immediately after HIV infection, initiating treatment during acute infection can also reduce the risk of HIV-1 transmission substantially.

Need for updated recommendations

- The use of HIV-1 Western blot in the previous algorithm misclassifies the majority of HIV-2 infections.
- Correct identification of HIV-2 infections is challenging, but accurate diagnosis of HIV-2 is clinically important because some antiretroviral agents are effective against HIV-1 (including nonnucleoside reverse transcriptase inhibitors and some protease inhibitors) and not effective against HIV-2.
- Considerable serologic cross-reaction occurs between HIV-1 and HIV-2, but screening exclusively with tests for HIV-1 antibodies failed to detect 15 to 53% of HIV-2 infections.
- As of May 2014, all FDA approved 3rd and 4th generation immunoassays incorporate specific antigens to detect antibodies directed against both HIV-1 and HIV-2.
- When HIV-1/HIV-2 immunoassays are repeatedly reactive, CDC’s previous recommendations advised specific testing for HIV-2 for specimens with negative or indeterminate HIV-1 Western blot results. However, studies published in 2012 and 2011 showed that the HIV-1 Western blot was interpreted as positive for HIV-1 in 46% to 85% of specimens from persons found to be infected with HIV-2, resulting in incorrect or delayed diagnosis.

Recommended HIV Testing Algorithm

- 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 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.

RECOMMENDED HIV TESTING ALGORITHM

Recommended HIV Testing Algorithm

- Laboratories should conduct 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 specimens that are nonreactive on the initial immunoassay.
- Rationale: Initial testing with a 4th generation antigen/antibody combination immunoassay detects more acute HIV-1 infections than initial testing with a 3rd generation antibody immunoassay and identifies comparable numbers of established HIV-1 and HIV-2 infections, with comparable specificity.
Recommended HIV Testing Algorithm

- 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.
- 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.

Recommended HIV Testing Algorithm

- 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 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 antibodies confirmed by HIV-1 NAT.
- A negative HIV-1 NAT result and nonreactive or indeterminate HIV-1/HIV-2 antibody differentiation assay result indicates a false-positive result on the initial immunoassay.

Recommended HIV Testing Algorithm

- Laboratories should use this same 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.
- Rationale:
  - Previously, supplemental testing (HIV-1 Western blot or HIV-1 IFA) was recommended after a reactive rapid HIV test regardless of the result of the initial laboratory immunoassay.
  - This was based on observations of some false-negative results from earlier generations of immunoassays (no longer commercially available in the US) that became reactive later during seroconversion than rapid HIV antibody tests.
  - With the recommended algorithm, the FDA-approved laboratory-based antigen/antibody combination immunoassays detect HIV infections earlier during seroconversion than any of the rapid HIV tests available in the US (as of May 2014), including the rapid HIV-1/HIV-2 antigen/antibody combination test.
  - Therefore, no supplemental testing is required for specimens that are nonreactive on the initial immunoassay in the recommended algorithm.

Recommended HIV Testing Algorithm

- Rationale:
  - Use of the HIV-1/HIV-2 antibody differentiation assay after a reactive initial 4th generation HIV-1/HIV-2 antibody immunoassay detects HIV-1 antibodies earlier than the HIV-1 Western blot, reduces indeterminate results, and identifies HIV-2 infections.
  - Turnaround time for test results is shorter and the cost is lower for the HIV-1/HIV-2 antibody differentiation assay compared with the HIV-1 Western blot.
  - Available evidence is sufficient to recommend specific additional testing, without clinical follow-up, for specimens that are dually reactive for HIV-1 and HIV-2 antibodies on the differentiation immunoassay.

Recommended HIV Testing Algorithm

- Rationale: HIV-1 NAT results can distinguish acute HIV-1 infection from false positive initial immunoassay results in specimens with a reactive antigen/antibody immunoassay and a nonreactive HIV-1/HIV-2 antibody differentiation assay result. HIV-1 NAT does not detect HIV-2, and no HIV-2 NAT is FDA approved. Available evidence is insufficient to recommend testing for acute HIV-2 infections after a nonreactive HIV-1 NAT result.

ALTERNATIVE TESTING SEQUENCES WHEN TESTS IN THE RECOMMENDED ALGORITHM CANNOT BE USED
Alternative Testing Sequences

• Use of a 3rd generation HIV-1/HIV-2 antibody assay instead of a 4th generation antigen/antibody combination immunoassay as the initial test: perform subsequent testing as specified in the recommended algorithm.

• Limitations: This alternative will miss some acute HIV-1 infections in antibody-negative persons that would be detected by 4th generation antigen/antibody combination immunoassays.

Alternative Testing Sequences

• Use of the HIV-1 Western blot of HIV-1 IFA as the second test in the algorithm instead of an HIV-1/HIV-2 antibody differentiation immunoassay:
  • if the test results are negative or indeterminate, perform HIV-1 NAT
  • If HIV-1 NAT is negative, perform HIV-2 antibody immunoassay.

Limitations: This alternative might misclassify some HIV-2 infections as HIV-1, requiring larger number of tests, and increases turnaround time for test results.

Alternative Testing Sequences

• Use of HIV-1 NAT as the second test instead of an HIV-1/HIV-2 antibody differentiation immunoassay:
  • If HIV-1 Nat result is negative, perform an HIV-1/HIV-2 antibody differentiation immunoassay or other FDA approved HIV-1 supplemental antibody test.
  • If result of an HIV-1 supplemental antibody test is nonreactive or indeterminate, perform an HIV-2 antibody test.

Limitations: This alternative fails to distinguish acute HIV-1 infection from established infection, increases turnaround time for test results and incurs additional costs.

Alternative Testing Sequences

• Use of HIV-1 NAT (or pooled HIV-1 NAT) after a nonreactive 3rd or 4th generation immunoassay result:
  • A reactive NAT result provides evidence of acute HIV infection but false-positives occur.
  • Follow-up testing to document seroconversion should be conducted if a laboratory HIV diagnosis is based on the result of HIV-1 NAT only.

Limitations: No HIV-1 Nat is FDA approved for pooled testing for HIV diagnosis. Individual or pooled HIV-1 Nat n detect acute infections not detected by a 4th generation immunoassay, but occasionally produces a false-positive result, requires more tests on each specimen, increases turnaround time for test results, and is more costly than the recommended algorithm.

Limitations

• No diagnostic test or algorithm can be completely accurate in all cases of HIV infection. Rare instances have been reported of persons who remained persistently negative for antibodies despite RNA.
• False positive HIV tests – specimen mix up, mislabeling, autoimmune disorders. Inconsistent or conflicting test results should be investigated with follow up testing on a newly collected specimen.
• A small percentage of specimens produce results that are undifferentiated (dualy reactive for HIV-1 and HIV-2 antibodies) on the HIV-1/HIV-2 antibody differentiation assay after completing all testing procedures recommended by the manufacturer.
Limitations

- None of the assays in the updated recommended algorithm are FDA approved for use with oral fluid or dried blood specimens. Laboratories should follow the 1989 recommendations for using the HIV-1 immunoassay and HIV-1 Western blot approved by the FDA for these specimen types.
- The recommended algorithm has not been evaluated in persons taking ART for preexposure or postexposure prophylaxis. Occurrence of delayed seroconversion have been reported in persons taking ART for preexposure and postexposure prophylaxis. As of May 2014, data are insufficient to determine whether additional follow up testing might be indicated for persons taking ART.

Limitations

- The recommended algorithm has not been evaluated in specimens from persons with long-term HIV suppression from antiretroviral therapy. Studies document that antibody levels diminish, some immunoassays become nonreactive, and the HIV-1 Western blot reverts from positive to indeterminate in a small number of patients who maintain undetectable levels of HIV, especially after antiretroviral therapy initiated early during the acute phase of the infection.

Limitations

- The recommended algorithm increases the ability to detect acute HIV-1 infection, but no laboratory assay can detect HIV infection immediately after it is acquired. The duration of the eclipse period between infection and the appearance of HIV RNA is not well defined from clinical studies and likely varies with the infection route, inoculum size, and sensitivity of the NAT used to detect HIV-1 nucleic acids.

REPORTING HIV TEST RESULTS

Reporting HIV test results

- Several elements of reports of test results and interpretation can help guide persons who ordered the HIV test to avoid interpretative errors.
- Reports should specify:
  - All assays that were used
  - The results of each assay
  - Interpretation of the results
  - Any additional testing that is recommended using existing specimen or new specimens that should be submitted
  - If alternatives to the recommended assays or algorithm sequence were used, the assays that were used and limitation of these tests or sequence compared with the recommended algorithm

Reporting results
QUESTIONS
For more information:
http://www.cdc.gov/hiv/testing/lab/guidelines