STATE OF MICHIGAN
DEPARTMENT OF HEALTH AND HUMAN SERVICES
LANSING

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# SELECTING APPROPRIATE HIV DIAGNOSTIC TESTS

Guidance from the Michigan Department of Health & Human Services/HIV Surveillance Program

With the release of improved HIV detection tests, we now have the ability to detect HIV infection earlier and identify patients in the acute phase when high levels of virus increase the likelihood of transmitting infection. The recommendations below will assist with- earlier detection of HIV and therefore the increased possibility of reducing transmission.

**Key Points**:

- Initial HIV screens should detect both antigen and antibody
- Two different reactive HIV tests may make an HIV diagnosis. If the second test is nonreactive or indeterminate, a third test must be run
- All initial reactive screening tests as well as all subsequent tests (regardless of the result) run must be reported to the health department. See contact person, page 3.

**Testing Steps**: (See generations of assays explained later in document)

Step 1: Initial screening test

- ✓ **Best: 4th generation antigen/antibody assay. Performed in lab or rapid test**
- ✓ Good: 3rd generation antibody assay that detects both IgG and IgM
- ✓ Acceptable: 2nd generation IgG antibody assay. Includes several rapid tests that do not detect HIV antigen or IgM antibody

Step 2: Supplemental test

- ✓ **Best: HIV-1/HIV-2 antibody type differentiating assay** (e.g. *Geenius*)
- ✓ Good: Western blot or IFA (being phased out)
- ✓ Acceptable: 2nd immunoassay, different from first. Advise further blood testing with Standard Laboratory Algorithm*

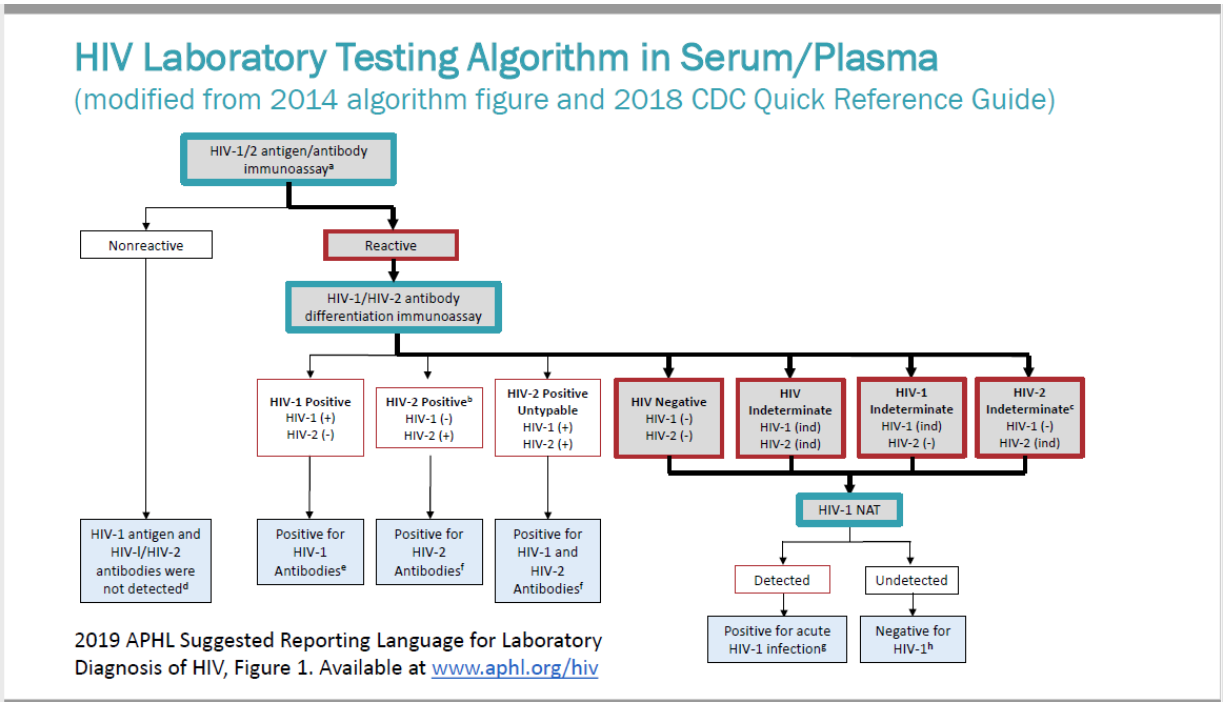
Step 3 if Discordant Results: If outcome of Step 1 is a reactive result and outcome of Step 2 is a negative or indeterminate result, a third definitive test is needed

- ✓ **Best: Nucleic Acid Test (NAT, RNA or DNA test), qualitative or quantitative (viral load)**
- ✓ No longer recommended but may be acceptable: repeat testing at a later date. Waiting to repeat testing may delay diagnosis and not resolve discordant results.

Questions: Marianne O'Connor
248-424-7922
oconnorm1@michigan.gov
Website: http://www.michigan.gov/hivstd

More guidance on following pages

*Standard Laboratory Algorithm:




Details:

1. **Initial HIV screens should detect both antigen and antibody.** These so called 4th generation screens include laboratory based versions and the Determine Combo rapid Ag/Ab test. The automated BioPlex screen discriminates between antigen and antibody as well as differentiates between HIV-1 and HIV-2 antibody at the same time. When a 4th generation test that detects both HIV antigen and antibody is not available, a 3rd generation HIV screen that detects both IgG and IgM HIV antibodies is acceptable but may miss diagnosing some recently infected persons.

2. The following laboratory tests should not be ordered as initial screening tests:
   a. HIV-1/HIV-2 antibody differentiating tests such as Bio-Rad ***Geenius*** *Supplemental Assay.* These laboratory tests are poor choices for an initial screening test as they do not detect either HIV antigen or IgM antibody, expressed during early infection. These tests are excellent choices for the second (confirmatory) test in a testing algorithm, following a reactive immunoassay screen.
   b. **Western blot**: This test is not a screening test. It is no longer a recommended confirmatory test following a reactive immunoassay and has largely been replaced by antibody differentiating tests in the standard laboratory algorithm. Antibody differentiating tests detect infection earlier than the Western blot and can identify and differentiate HIV-1 and HIV-2 in a single step.

3. **A reactive screen followed by a negative or indeterminate antibody differentiation test or Western blot must be followed up with a nucleic acid test (NAT)** to confirm or rule out infection. The HIV-1 NAT detects the RNA of the HIV virus itself and may be either qualitative (HIV detected/not detected) or quantitative (HIV viral load). At this time, some laboratories do not automatically perform a NAT to sort out conflicting test results; **the physician must order the follow-up test, usually plasma.** Some laboratories do run all 3 tests on the initial specimen when indicated.
Reactive initial screens that are followed by negative results on subsequent tests (false positives) are occasionally seen in pregnant patients, those with autoimmune disorders or those with other infections. Reactive initial screens that are followed by a negative second test and positive NAT are likely to be newly infected individuals with very high viral loads.

4. **Dual Immunoassays**: Two reactive immunoassays/screens that are "different" are now considered diagnostic of HIV infection. "Different" means the two tests are not testing for the same things, or are using different methods. Using two different manufacturers is sufficient. The assays may be rapid tests or conventional laboratory based assays.
    a. Following a reactive rapid immunoassay screen, blood sent to a laboratory for confirmatory testing should start with the antigen/antibody screen, NOT with a confirmatory antibody differentiation test or Western blot.
    b. Rapid screening tests are usually 2nd or 3rd generation tests, meaning they only detect antibodies. (See rapid test specifics later in this document.) An exception:
    c. ***Determine Ag/Ab Combo* rapid test:** 4th generation test; detects antibodies to HIV-1 and HIV-2, as well as the p24 antigen of HIV-1. The test goes one step further than other rapid tests and most 4th generation tests, letting the tester know whether antigen is reactive, antibody is reactive, or both. It is considered to be the most sensitive of the rapid screens but **testing on finger stick whole blood specimens is not as sensitive near the time of infection as Ag/Ab lab tests performed on plasma or serum.**
    d. Follow up with laboratory testing with either:
        i. Standard laboratory testing: 4th generation antigen/antibody screen, HIV-1/HIV2 antibody differentiating test and a NAT if needed.
        ii. Viral load, CD4 and antiviral resistance assay

5. None of the assays in the Standard Laboratory Algorithm are FDA-approved for use with oral fluid or dried blood spot specimens. Laboratories will use the HIV-1 immunoassay and Western blot approved for those specimen types. **The MDHHS state laboratory no longer tests oral specimens.**
6. Reporting HIV infections to the health department:

- All initial reactive screening tests as well as all subsequent tests (regardless of the result) must be reported to the health department. Report each separate analyte detected by the test as reactive or nonreactive:
    - *Determine Ag/Ab Combo* rapid test: report both antigen and antibody reactivity
    - *BioPlex Ag/Ab Assay* lab-based test: Report antigen, antibody to HIV-1, antibody to HIV-2
- Contact Laboratory Reporting Coordinator for reporting options:
  Erin Crandell-Alden, 517-284-4918, crandelle@michigan.gov
- Feel free to attach lab results to an HIV Case Report Form whenever there is any question about laboratory results.

**Generations of HIV Immunoassays**:

- First generation: whole viral lysate detects IgG antibody. Includes HIV-1 Western blot and HIV-1 IFA.
- Second generation: improved specificity over first generation tests by adding recombinant proteins or synthetic peptides. Detects IgG antibody to HIV-1 and (sometimes) HIV-2. Includes the HIV-1 EIA and these rapid tests:
    - DPP HIV 1/2 rapid test
    - HIV 1/2 Stat-Pak rapid test
    - Reveal G4 rapid test
    - SURE CHECK rapid test
    - Geenius antibody differentiation test (supplemental rapid test, not a screen)
- Third generation: detects IgG and earlier IgM antibody to HIV 1 and 2. Includes the following laboratory tests and rapid tests:
    - Bio-Rad GS HIV-1/2 Plus O lab test
    - Siemens Advia Centaur 1/O/2 lab test
    - Ortho Vitros HIV 1+2 lab test
    - Avioq HIV-1 Microelisa lab test
    - VITROS Anti HIV 1+2 lab test
    - Unigold Recombigen rapid test
    - INSTI rapid test
    - OraQuick ADVANCE rapid test
- Fourth generation: detects IgG and IgM antibody to HIV-1 and HIV-2, plus HIV-1 p24 antigen. Includes:
    - Abbott Architect Ag/Ab Combo lab test
    - ADVIA Centaur Ag/Ab Combo CHIV lab test
    - Bio-Rad Ag/Ab Combo lab test
    - Elecsys HIV combi PT lab test
    - BioPlex Ag-Ab Assay: automated lab test discriminates between antigen and antibody as well as differentiates between HIV-1 and HIV-2 antibody at the same time. Therefore, some refer to this test as a "5$^{th}$ generation" screen.
    - Determine Combo Ag/Ab rapid test (goes a step further than other rapid tests to discriminate antigen from antibody). When used with finger stick whole blood specimens, it is not as sensitive near the time of infection as Ag/Ab lab tests performed on plasma or serum.

**Final Notes**:

- MDHHS does not endorse any particular test manufacturer and only includes brand names here to clarify current testing options
- Antibody-only tests do not detect infection in ~10% of infected persons. These acute infection cases tend to have high viral loads and thus are at highest risk of transmitting the virus to others.

**Helpful links**:

Updated Recommendations from CDC: Laboratory Testing for the Diagnosis of HIV Infection: https://stacks.cdc.gov/view/cdc/23447

Advantages and disadvantages of FDA-approved HIV immunoassays used for screening https://www.cdc.gov/hiv/pdf/testing/hiv-tests-advantages-disadvantages_1.pdf