SELECTING APPROPRIATE HIV DIAGNOSTIC TESTS
Guidance from the Michigan Department of Community Health/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. Please follow the recommendations below for 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

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 most 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. Multispot, Geenius)
  - Good: Western blot or IFA (now being phased out)
  - Acceptable: 2nd immunoassay, different from first. Advise further blood testing with New 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), 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
Details:

1. **Initial HIV screens should detect both antigen and antibody.** These so called 4th generation screens include laboratory based versions and the rapid Determine Ag/Ab test. 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 tests should not be ordered as initial screening tests:**
   a. HIV-1/HIV-2 antibody differentiating tests such as Multispot or Geenius. 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 test in a testing algorithm, following a reactive immunoassay screen.
   b. **Western blot:** This test is not a screening test. It was long-used as the second test in the old laboratory algorithm, following a reactive immunoassay. It is no longer a recommended test and is slowly being replaced by HIV-1/HIV-2 antibody differentiating tests in the standard laboratory algorithm. The Multispot and Geenius tests detect infection earlier than the Western blot and can identify and differentiate HIV-1 and HIV-2 in a single step. They also have the added advantage of being rapid tests.

3. **A reactive screen followed by a negative or indeterminate Multispot, Geenius 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, most laboratories do not automatically perform a NAT to sort out conflicting test results; the physician must order the follow-up test. A few laboratories will 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. A reactive rapid immunoassay screen may be followed by a laboratory based immunoassay such as the Multispot, Geenius or Western blot. **This is the only time it is appropriate to order a stand-alone Multispot, Geenius or Western blot from a laboratory.**

   b. Most rapid screening tests are 2nd generation, meaning they only detect HIV-1 IgG antibodies. Exceptions include the 3rd generation Unigold Recombigen and:

   c. **Determine Ag/Ab Combo rapid test:** 4th generation; 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 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 data from widespread use on whole blood are needed to confirm this.

   d. Follow up with laboratory testing with either:

      i. Standard laboratory testing: 4th generation screen, HIV-1/HIV2 differentiating test and a NAT if needed.

      ii. Viral load, CD4 and antiviral resistance assay

5. **Do not confuse the p24 antigen test result** seen on an antigen/antibody screen reports with the traditional p24 with neutralization test that is rarely done now. Do not report a “p24 antigen” test result to the health department from an antigen/antibody screen; instead simply report “antigen/antibody reactive” results. When running the Determine rapid test that goes a step further and distinguishes antigen from antibody reactivity, report each component (antigen and antibody) as reactive or nonreactive.

6. None of the assays in the New 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.

7. **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. Contact ELR Coordinator for reporting options:

     Erin Crandell-Alden, 517-335-9464, 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 most rapid tests:
  - Multisport HIV-1/HIV-2
  - OraQuick Advance
  - Clearview Complete
  - MedMira Reveal G3
  - INSTI
  - Chembio Stat Pak
- Third generation: detects IgG and earlier IgM antibody to HIV 1 and 2. Includes 3 laboratory tests and one rapid test:
  - Bio-Rad GS HIV-1/2 Plus O
  - Siemens Advia Centaur 1/O/2
  - Ortho Vitros HIV 1+2
  - Unigold Recombigen 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
  - Bio-Rad Ag/Ab Combo lab test
  - Determine Combo Ag/Ab rapid test (goes a step further to discriminate antigen from antibody)

Final Notes:

- MDCH does not endorse any particular test manufacturer and only includes brand names here to clarify the 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::

Advantages and disadvantages of FDA-approved HIV immunoassays used for screening
[http://www.cdc.gov/hiv/pdf/testing_AdvDisadvHIVtesting.pdf](http://www.cdc.gov/hiv/pdf/testing_AdvDisadvHIVtesting.pdf)

MDCH HIV case report forms and instructions:
[http://www.michigan.gov/mdch/0,4612,7-132-2940_2955_2982_46000_46002---,00.html](http://www.michigan.gov/mdch/0,4612,7-132-2940_2955_2982_46000_46002---,00.html)