

## LAB RESOURCE GUIDE FOR HIV, SYPHILIS, HEPATITIS B, AND HEPATITIS C INFECTION DURING PREGNANCY

Laboratory test results for HIV, syphilis, hepatitis B, and hepatitis C must be interpreted with caution. A single positive or reactive test result does not definitely indicate current infection. The entire battery of test results must be considered together. Birthing people are screened for all four of these infections in their first trimester, in the third trimester, and tested again at delivery (if status is unknown or if the birthing person has high risk factors). Infants have specific tests indicated.

### Key Points

#### Test results that point to infection:

- HIV
  - Positive HIV screen plus positive confirmatory test, or
  - Detectable HIV Nucleic Acid Test (NAT, RNA, DNA, PCR, viral load).
- Syphilis
  - Positive treponemal test (EIA, CIA, TPPA, TPHA, FTA-ABS) along with positive nontreponemal test (RPR, USR, VDRL).
- Hepatitis B
  - Positive hepatitis B surface antigen (HBsAg).
  - Positive hepatitis B virus DNA (HBV DNA).
- Hepatitis C
  - Positive HCV antibody (anti-HCV test) and detectable HCV nucleic acid test (HCV NAT, HCV RNA, HCV Viral Load).

#### Child versus adult testing:

An infant whose birthing person is infected with HIV or syphilis may carry the birthing person's antibodies for up to the first two years of life, so antibody-based tests reflect the birthing person's infection status rather than that of the infant. Infants should not be tested for hepatitis B before nine to 12 months of age. Infants should not be tested for hepatitis C before 2 months of age.

#### Appropriate testing of infants:

- HIV: HIV RNA/DNA Nucleic Acid Test (NAT, PCR, viral load).
- Syphilis: same as adult tests (treponemal plus nontreponemal test) with addition of the syphilis IgM western blot test if either of the first tests is positive.
- Hepatitis B: HBsAg and hepatitis B surface antibody (anti-HBs) testing 3-6 months after receiving hepatitis B (HepB) vaccine and hepatitis B immune globulin (HBIG) at birth along with at least two additional doses of HepB vaccine.
- Hepatitis C:
  - Infants who are born to persons with current (has detectable HCV RNA) or probable (anti-HCV reactive, HCV RNA results are not available) HCV infection should be tested between ages 2-6 months with an HCV RNA test.
  - Infants/children who are perinatally exposed to HCV but have not been previously tested should receive HCV RNA testing if they are ages 7-17 months.
  - Infants/children who are perinatally exposed to HCV but have not been previously tested should receive an anti-HCV test with reflex to HCV RNA test if they are age 18 months or older.

**Refer to the pages below for more detail on the testing of each infection. Reach out to the Michigan Department of Health and Human Services (MDHHS) surveillance staff with questions about interpreting lab results:**

- HIV: 313-456-1586
- Syphilis: 313-456-1586
- Hepatitis B: 517-242-8319
- Hepatitis C: 517-335-8165

## Human Immunodeficiency Virus (HIV)

### Important notes:

Two positive test results in a testing sequence are generally needed to confirm HIV infection. Never infer a confirmed infection from a single positive test result. Reactive screens can occasionally be false positive results therefore all positive screens require confirmation testing.

Newborns may carry their HIV positive birthing person's antibodies for the first two years of life. Positive antibody tests on infants do not necessarily indicate infection. HIV RNA or DNA testing must be run on children under the age of two.

### Screening: usually HIV antigen and antibody screen

- May be rapid field test or a complex test performed in a laboratory.
- Laboratory test is better/preferred over rapid test performed outside the laboratory.
- False positive screens do occur rarely. All reactive screens must be confirmed with a second, confirmatory test.
- False negative screen results occur if there has not been enough time since infection (two to four weeks, depending on the test).

### Confirmatory testing: usually the HIV-1, 2 Antibody Differentiation test

- Two results for antibodies to HIV-1 and HIV-2 obtained. Expect HIV-2 to be negative as it is extremely rare in the United States.
- HIV-1 antibody reactive results confirm infection. If HIV-1 antibody result is negative, a false positive screen is a high possibility and HIV-1 RNA testing is needed to sort out the results.
- May still see positive/negative results for old, outdated confirmatory test (HIV-1 Western Blot)

### Rare third test: HIV-1 RNA (also referred to as NAT, PCR, viral load)

- Test detects the nucleic acid of the HIV virus itself.
- Usually run to sort our results when screening and confirmatory tests do not agree.
- Occasionally run in place of the confirmatory Antibody Type Differentiation test.
- Should be used to screen for infection in children under the age of two.

### Summary of HIV testing sequence:

<i>Test 1</i>	<i>Test 2</i>	<i>Test 3 (only if needed to sort out test 1 and 2)</i>	<i>Conclusion</i>
HIV screen	HIV1, 2 Antibody Differentiation confirmatory test	HIV-1 RNA (Nucleic Acid Test)	
Positive	Positive for HIV-1		Infection confirmed
Positive	Negative for HIV-1	RNA not detected	Infection NOT confirmed; screen was false positive
Positive	Negative for HIV-1	RNA detected	Infection confirmed. Likely acute infection.

## Syphilis

Syphilis is a sexually transmitted infection with the spirochete bacterium *Treponema Pallidum (TP)*. It can be passed from birthing person to child in utero. The diagnosis of syphilis is based on both clinical findings and serologic tests. Presumptive diagnosis requires the use of two serologic tests: Treponemal tests that detect antibodies to the TP organism and a non-Treponemal test, which measures a non-specific antibody to Treponemal infection.

Non-Treponemal tests will have accompanying titers if found to be positive. A titer indicates the concentration of antibodies. It is the highest dilution at which antibodies are detected. For example, a titer of 64 means that antibodies were detected when the patient's serum was diluted up to 1:64. A positive titer indicates active syphilis and follow up titers are performed to monitor response to therapy.

**The sequence of tests varies with the laboratory: some labs begin with a non-Treponemal test and others begin with a Treponemal antibody test.**

- Common non-Treponemal tests are Rapid Plasma Reagin (RPR), Unheated Serum Reagin (USR), or Venereal Disease Research Laboratory (VDRL) tests.
- Common Treponemal tests that detect antibodies to TP: various immunoassays (EIA, CIA), Particle Agglutination (TPPA), Hemagglutination (TPHA), Fluorescent Treponemal Antibody Absorbed (FTA-ABS) test, and the IgM Western Blot test.

### Important notes:

- False negative syphilis test results occur if there has not been enough time since infection (three to six weeks) to develop antibodies.
- Treponemal antibodies persist for life, thus Treponemal tests cannot distinguish between recent, active infection and previously treated or old, non-contagious infection.
- False positive Treponemal test results occur with other infections, autoimmune diseases, immunizations, pregnancy, injection-drug use, and with advancing age.
- False positive non-Treponemal results occur with tuberculosis, rickettsial diseases, endocarditis, pregnancy, or any condition involving tissue destruction.

### Syphilis testing in children:

- The persistence of Treponemal antibodies up to 18 months of age may point to congenital infection in the asymptomatic child and not necessarily indicate exposure by another means, including sexual abuse.
- The syphilis IgM Western Blot test is run on babies who are positive for either the Treponemal or non-Treponemal test to distinguish between the baby and birthing person's antibodies. A reactive result for the baby on the IgM Western Blot test suggests congenital or primary infection with TP, the syphilis organism.
- Diagnosis and management of congenital syphilis should be based on the birthing person's history, clinical findings, and non-Treponemal testing.

### Syphilis Continued

**Syphilis (Treponema Pallidum / TP) test interpretation:**

<i>Treponemal test</i>	<i>Non-Treponemal test</i>	<i>Possible interpretations</i>
EIA, CIA, TPPA, TPHA, FTA-ABS, etc.	RPR, USR, VDRL	
Nonreactive	Nonreactive	Uninfected or very early syphilis before seroconversion.
Reactive	Nonreactive	Prior treated syphilis, untreated syphilis or false positive Treponemal test. Baby: run IgM Western Blot test.
Reactive	Reactive	Active syphilis, recently treated syphilis, or treated syphilis with persistent titers.
Nonreactive	Reactive	False positive non-Treponemal test. Baby: run IgM Western Blot test.

**The clinical manifestations of syphilis are classified by stages to help guide treatment and follow up:**

The stages are primary, secondary, latent, and late or tertiary syphilis, including neurosyphilis and cardiovascular syphilis. Information that is needed to determine the stage includes a thorough medical and sexual history which must include a history of syphilis testing and treatment.

## Hepatitis B Virus (HBV)

Hepatitis B testing involves measurement of different HBV-specific markers for antigens and antibodies. Different combinations of test results are used to determine whether a patient has acute or chronic HBV infection, is immune to HBV because of prior infection or vaccination, or is susceptible to infection

### The four most common HBV tests are:

- Hepatitis B surface antigen (HBsAg): A positive result indicates the person is infectious and carries the virus in their blood. It is positive during both acute and chronic infection.
- Hepatitis B surface antibody (anti-HBs): A positive result indicates the person has recovered from HBV infection and has immunity to the virus. Anti-HBs positive result also develops in a person who has been successfully vaccinated against HBV.
- Hepatitis B core antibody – IgG/IgM (anti-HBc Total): A positive result appears at the onset of symptoms in acute HBV infection and persists for life. The presence of anti-HBc total indicate either previous or ongoing infection with HBV.
- Hepatitis B IgM antibody to core antigen (IgM anti HBc): A positive result indicates recent/acute infection with HBV (≤ six months).

Additionally, hepatitis B Virus DNA (HBV DNA) measures viral load in the blood of infected individuals and guides treatment decisions. A positive result indicates the person is infectious.

### Building immunity in susceptible people:

- Hepatitis B (HepB) vaccine: Triggers the natural production of surface antibodies (anti-HBs) to the virus. These antibodies confer immunity while anti-core antibodies do not.
- Hepatitis immune globulin (HBIG): Passive administration of hepatitis antibodies to provide short-term protection from infection.

### False positive and negative results occasionally occur:

- False positive HBsAg results occur if tested too soon after hepatitis B vaccine is given.
- Newborns may have false positive anti-HBs results because they were tested too soon after receiving the HBIG.
- Infants should not be tested for HBsAg and anti-HBs before nine to 12 months of age.

### Hepatitis B screening serologic results

HBV surface HBsAg	HBV core antibody Anti-HBc Total	HBV core Anti-HBc IgM	HBV surface Anti-HBs	Interpretation
Negative	Negative	--	Negative	Susceptible
Negative	Positive	--	Positive*	Immune (natural infection)
Negative	Negative	--	Positive*	Immune (due to vaccination)
Positive	Positive	Negative	Negative	Chronic HBV infection
Positive	Positive	Positive	Negative	Acute HBV infection

\*Seroprotective levels of >10 mIU/mL

## Hepatitis C Virus (HCV)

### Important notes:

To confirm hepatitis C virus, two positive test results in a testing sequence are needed. A confirmed infection should not be inferred from one single positive test result. Reactive antibody tests can occasionally be false positive results therefore all positive antibody tests require confirmation testing.

- Hepatitis C Antibody test: A non-reactive or negative antibody test indicates that the person is not currently infected with the hepatitis C virus. A reactive or positive antibody test indicates a person has been infected with the hepatitis C virus at some point in time. A reactive antibody test does not mean a person is currently infected and a follow up test is needed to confirm infection. Once a person has been infected with hepatitis C, they will always have antibodies in their blood.
- Hepatitis C RNA test: A non-reactive or negative RNA test along with a reactive or positive antibody test result indicates the person was infected with the hepatitis C virus, but the virus is no longer in the person's body because the individual was cured with treatment or cleared the virus naturally. A reactive or positive RNA test result indicates the person is infectious and carries the virus in the blood.

Newborns may carry antibodies from their birthing person up until the age of 18 months. Positive antibody tests on infants do not necessarily indicate infection. Infants should not be tested for HCV before the age of two months.

Summary of HCV testing results:

<b>Test outcome</b>	<b>Interpretation</b>	<b>Further actions</b>
HCV antibody nonreactive	No HCV antibody detected	Sample can be reported as nonreactive for HCV antibody. No further action required.  If recent exposure in person tested is suspected, test for HCV RNA*
HCV antibody reactive	Presumptive HCV infection	A repeatedly reactive result is consistent with current HCV infection, or past infection that has resolved, or biological false positivity for HCV antibody. Test for HCV RNA to identify current infection.
HCV antibody reactive, HCV RNA detected	Current HCV infection	Provide person tested with appropriate counseling and link person tested to care and treatment. **
HCV antibody reactive, HCV RNA not detected	No current HCV infection	No further action required in most cases.  If distinction between true positivity and biologic false positivity for HCV antibody is desired, and if sample is repeatedly reactive in the initial test, test with another HCV antibody assay.  In certain situations, *** follow up with HCV RNA testing and appropriate counseling.

\*If HCV RNA testing is not feasible and person tested is not immunocompromised, do follow up testing for HCV antibody to demonstrate seroconversion. If the person tested is immunocompromised, consider testing for HCV RNA.

\*\*It is recommended before initiating antiviral therapy to retest for HCV RNA in a subsequent blood sample to confirm HCV RNA positivity.

\*\*\*If the person tested is suspected of having HCV exposure within the past six months, or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.