

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 mother's antibodies for the first 2 years of life. Positive antibody tests on infants do not necessarily indicate infection. HIV RNA or DNA testing must be run on children less than 2 years of age.

- **Adult screening:** usually HIV antigen & antibody screen
 - o May be rapid field test or a complex test performed in a laboratory
 - o Laboratory test is better/preferred over rapid test performed outside the lab
 - o False positive screens do occur rarely. All reactive screens must be confirmed with a second, confirmatory test.
 - o False negative screen results occur if there has not been enough time since infection (2-4 weeks, depending on test)
- **Confirmatory Testing:** usually the HIV-1,2 Antibody Differentiation test
 - o Two results for antibodies to HIV-1 and HIV-2 obtained. Expect HIV-2 to be negative as it is extremely rare in the U.S.
 - o HIV-1 antibody reactive result confirms 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
 - o May still see pos/neg results for old, outdated confirmatory test: HIV-1 Western blot
- **Rare 3rd test:** HIV-1 RNA (also referred to as NAT, PCR, viral load)
 - o Test detects the nucleic acid of the HIV virus itself
 - o Usually run to sort out results when screening and confirmatory tests do not agree
 - o Occasionally run in place of the confirmatory Antibody Type Differentiation test
 - o Should be used to screen for infection in children less than 2 years of age

Summary of HIV Testing Sequence:

Test 1	Test 2	Test 3: if needed to sort out Tests 1&2	Conclusion
HIV screen	HIV 1,2 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

Hepatitis B Virus (HBV):

Important Notes:

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 as a result of prior infection or vaccination or is susceptible to infection.

The 4 most common HBV tests are:

- **Hepatitis B surface antigen (HBsAg):** A positive result indicates the person is infectious and carries the virus in the 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 indicates 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 (≤ 6 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 9-12 months of age.

Hepatitis B Screening Serologic Results

HBV surface antigen	HBV core antibody – IgG/IgM	HBV core antibody - IgM	HBV surface antibody	Interpretation
HBsAg	Anti-HBc Total	Anti-HBc IgM	Anti-HBs	
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

Syphilis

Syphilis is a sexually transmitted infection with the spirochete bacterium *Treponema pallidum* (TP). It is also passed from mother to child in utero. The diagnosis of syphilis is based on both clinical findings and serologic tests. Presumptive diagnosis requires the use of 2 serologic tests: treponemal tests that detect antibodies to the TP organism as well as a nontreponemal test, which measure a non-specific antibody to treponemal infection.

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

The sequence of tests varies with the laboratory: some labs begin with a nontreponemal 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, IgM Western blot test etc.

Important Notes:

- False negative syphilis test results occur if there has not been enough time since infection (3-6 weeks) to develop antibodies.
- Treponemal antibody persists 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 in other with other infections, autoimmune disease, immunizations, pregnancy, injection-drug use and with advancing age.
- False positive nontreponemal results occur with tuberculosis, rickettsial diseases, endocarditis, pregnancy or any condition involving tissue destruction.
- Syphilis testing in children:
 - o The persistence of treponemal antibody 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.
 - o The syphilis IgM Western blot test is run on babies who are positive for either the treponemal or nontreponemal test to distinguish between the baby and mother's antibodies. A reactive result for the child on the IgM Western blot test suggests congenital or primary infection with TP, the syphilis organism.
 - o Diagnosis and management of congenital syphilis should be based on maternal history, clinical findings and nontreponemal and treponemal testing.

Syphilis (*T. pallidum*) Test Interpretation:

Treponemal Test	Nontreponemal Test	Possible Interpretations
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 titres
Nonreactive	Reactive	False positive nontreponemal test. Baby: run IgM Western blot test

The clinical manifestations of syphilis are classified by stages to help guide treatment and follow up: primary, secondary, latent, and late or tertiary syphilis, including neurosyphilis and cardiovascular syphilis. Information needed to determine stage includes a thorough medical and sexual history, including history of syphilis testing and treatment.