

Interpretation of Discordant/Incomplete Hepatitis B Serology

Hepatitis B laboratory studies are often difficult to interpret due to either incomplete testing or results that seem to be contradictory/discordant with each other (e.g. IgM anti-HBc (+) with anti-HBc (-)). The following scenarios will cover most of the frequently seen discordant laboratory results. If you have other scenarios that are not covered, or you just require further assistance, contact the Viral Hepatitis Surveillance Nurse.

IgM anti-HBc or Anti-HBc (+) reported alone:

- Request all additional hepatitis b testing performed including negative results.
 - If there are no additional tests available, determine if the patient had a discrete onset of symptoms in the last 6 months.
 - Not symptomatic
 - No need to report case. If an event already exists report to the state with a classification of “Does not meet criteria” no investigation is required.
 - Symptomatic
 - Additional testing is needed to verify patients Hepatitis B status. Either the patient’s primary care provider or the LHD via the State Lab of Public Health (SLPH)
<https://slph.ncpublichealth.com/virology-serology/hepatitis.asp> can order additional testing.
 - HBsAg, IgM anti-HBc, anti-HBc and anti-HBs
 - Using the SLPH form 3722 you will choose “Hepatitis B Virus (HBV) Monitor” selecting “Follow-up of person with previous positive test for HBsAg or history of Hepatitis B infection”
 - Case will be classified according to the test results.
 - If no additional testing can be performed or patient is lost to follow-up (LTF), change case classification to “Does not meet criteria”.
- Additionally, the IgM anti-HBc cannot be (+) when the anti-HBc is (-) since said IgM anti-HBc is a part of the anti-HBc. Likely, the IgM is a false positive. Request all additional HBV test results, if they are negative there is no need to report the case. If an event already exists report it to the state with a classification of “Does not meet criteria” no investigation is required.

HBV DNA resulted as less than the measurable range and reported with no indication of detection:

- Report does not indicate (+ or -)
 - Call the testing facility and/or laboratory to determine the qualitative result.
 - If you do not call either of the facilities treat the results as a positive HBV DNA.

HBsAg & anti-HBs (+) simultaneously or HBsAg (+) after the anti-HBs appears:

- Request all additional hepatitis b testing performed including negative results.
- Determine if the patient was recently vaccinated (within the last 18-20 days for regular vaccine and 52-60 days for dialysis doses).
 - If the patient was recently vaccinated please follow the instructions for “False Positive HBsAg”.
 - No history of recent vaccination, the event will need to be changed from a lab condition report to acute or chronic depending on the patients’ clinical presentation compared to the case definitions.
 - Patient is more than likely beginning the process of clearing the virus. Event will be reported and once HBsAg disappears (HBsAg clearance signifies immunity per guidelines) patient will no longer have any more HBV events in NC EDSS.

HBeAg (+) with HBsAg (-)

- The HBeAg represents heavy/active viral reproduction and should not be positive without a positive HBsAg which simply means the virus is present. If an HBeAg (+) with a HBsAg (-), it could represent a false positive HBeAg or a false negative HBsAg. False negative HBsAg’s can occur when levels of the surface antigen overwhelm the assay. This would most likely occur when there are high levels of viral reproduction which would result in a HBeAg (+), so re-testing would be in order. Unless of course, an anti-HBc (-) was documented at the same time indicating no exposure to hepatitis B and supporting the negative HBsAg result.

HBsAg versus HBsAg Neutralization Assay (NA)

- HBsAg is detected through the use of immunoassays that use anti-HBs to capture antigen in the sample. Like most infectious disease assays, nonspecific binding can and does occur. Manufacturers have determined a “cutoff value” that balances the necessary high sensitivity for detecting the antigen (or anti-HBs antibody) with the need to avoid false-positive results. Samples with signal values (S) above the “cutoff value” are called positive (S/C ratio >1). In the case of tests for antibodies to viral antigens (e.g., anti-HCV and anti-HIV), weakly positive results are often falsely positive and require confirmation using isolated viral antigens. In the case of

tests for HBsAg, manufacturers provide a neutralization test that can be used to confirm true positivity of the results. In this test, samples with S/C ratio >1 are incubated with anti-HBs; if HBsAg is truly present, the anti-HBs in the neutralization step blocks binding to the reagent antibodies, reducing or eliminating the signal from HBsAg (positive neutralization test result).

- A positive/confirmed neutralization test is considered the **definitive** test for HBsAg. Samples that are reactive by the screening test but negative (not confirmed) by the neutralization test are likely to contain cross-reactive antibodies or immunologic disorders. These unconfirmed HBsAg screening test results should be interpreted in conjunction with other HBV serological markers (eg, anti-hepatitis B surface antibody, anti-hepatitis B core total antibody).
- Patients who are HBsAg positive by the screening EIA but negative by the confirmatory NA should be considered negative for hepatitis B infection.

Please note that this guidance only applies to events with a negative HBsAg NA result. If the NA result is positive, if no NA result is received, or if it is not clear which testing method was used (EIA vs. NA), the HBsAg should be considered positive.

LHD responsibility/role:

- Whenever an HBsAg neutralization test is performed you will always have two results: HBsAg & HBsAg neutralization (often results days later).
 - If the HBsAg (-) and the laboratory runs the HBsAg neutralization test and results (+) that will be considered a false positive neutralization test (since the first HBsAg was (-) the neutralization test should never have been prompted)
 - Please document and/or request both results when reporting events to the state.

For more information/source: <http://clinchem.aaccjnls.org/content/52/8/1457>

HBsAg false positives

- For patients undergoing hemodialysis, higher vaccine dosages or an increased number of doses are recommended. One formulation of hepatitis B vaccine designed for hemodialysis patients and other immunocompromised adult (age ≥20 years) patients it contains an increased dosage and is administered in a 3-dose schedule (Recombivax HB, 40 µg/mL). The other available formulation of hepatitis B vaccine is administered at a double standard dosage in a 4-dose schedule for hemodialysis patients and other immunocompromised adult (age ≥20 years) patients (two Engerix-B, 20ug) administered in 1 or 2 injections.
- The need for booster doses will be assessed by annual testing for antibody to hepatitis B surface antigen (anti-HBs). A booster dose should be administered when anti-HBs levels decline. As a result, hemodialysis patients will receive numerous HBV vaccines.
 - (source: https://www.cdc.gov/dialysis/pdfs/vaccinating_dialysis_patients_and_patients_dec2012.pdf)
 - Obtain
 - Retesting 60 days after the date of the last vaccine administration date

- Transient HBsAg positivity can occur up to 18 days following vaccination (up to 52 days among hemodialysis patients) (source: <https://www.cdc.gov/mmwr/volumes/67/rr/rr6701a1.htm>)
- Prior HBsAg/HBV testing results 3 months prior to the positive
 - If unable to procure prior HBV serology or no prior HBV testing was performed, retesting 60 days after the date of the last vaccine will need to occur.
 - If HBV testing was performed and found to be negative retesting up 30 days after the date of the last vaccine will be sufficient.
- Vaccination record
- NC EDSS Documentation
 - Event will remain as a lab condition report
 - Document on the dashboard that the event is being worked up for a possible false positive HBsAg from a recent vaccination.
 - All vaccines given should be added to the vaccine package
 - Update lab results section with prior testing as well as repeat testing results

If follow-up testing is positive, please change the event to either acute or chronic and follow:

- Investigation of Suspected Healthcare Acquired Hepatitis B or Hepatitis C
- Management of exposure to HBsAg (+) & HCV (+) dialysis patients in a dialysis setting

Lost to follow up:

- When discordant labs are received, and the person cannot be located or has refused additional testing, they should be considered infectious if any of their testing includes a HBsAg, HBeAg or HB DNA (+) result and there is not overwhelming evidence, (at least 2 tests) that the positive (s) are erroneous.
 - Example:
 - HBV DNA (+) and HBsAg (-) and/or HBV DNA (-) and HBsAg (+) and retesting is not an option, the person should be considered infective.
 - Can often be the case when persons are on antiviral therapy which can often result in discordant test results such as HBV DNA (+) and HBsAg (-) and be actively infected. Case such as these will need special attention to determine if the person is potentially infective. If in doubt regarding discordant labs and the need for additional testing, please call the Viral Hepatitis Surveillance Nurse.