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 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 "Detected" or "Not Detected."
    - Call the testing facility and/or laboratory to determine the qualitative result.
        - If qualitative "Detected", consider this (+) HBV DNA. Follow the algorithm.
        - If "Not Detected", no other work up is needed. Report as "Does Not Meet Criteria"

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 20-30 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 patient's clinical presentation.

HBeAg (+) with HBsAg (-)

- The HBeAg represents heavy/active viral replication. 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 likely occurs when there are high levels of viral replication which would result in a HBeAg (+). In this scenario, repeating hepatitis B serology would be indicated.
  Two positive HBeAg results at least six months apart would meet criteria for Chronic HBV infection. Additional testing for HBV DNA would allow for clarification.

Positive HBsAg but Negative HBsAg Neutralization Assay (NA) (Reflex Testing)

- 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 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. These unconfirmed HBsAg screening test results should be interpreted in conjunction 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.
  - If the patient is asymptomatic, report as "Does Not Meet Criteria."
  - If the patient IS symptomatic, they may have had a recent exposure. Additional serologic testing is recommended.

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

HBsAg false positives

- Recent HBV Vaccination
    - Ask if patient has received HBV vaccine in the last 18-20 days (50-60 for dialysis patients)
    - If yes, recommend repeat testing on a date that surpasses the above ranges.
    - If patient is NOT symptomatic: No other serologies needed, although a Core HBV Ab negative result would confirm the false positive.

        - 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 are received.

- If follow-up testing is positive, please change the event to either acute or chronic and follow recommended protocol for further investigation
- If suspected Healthcare Acquired Infection, contact Healthcare Acquired Infection (HAI) at NCDHHS

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 positive HBsAg, HBeAg or HBV DNA result.
    - If there is no overwhelming evidence, (at least 2 tests) that the positives are erroneous or there is inability to perform additional testing that result was a false positive, the patient should be considered “Lost to Follow Up”.