| **Hepatitis B Surface Antibody (AUSAB)** |
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| **Purpose** | This procedure provides instructions for performing HEPATITIS B SURFACE ANTIBODY on the Abbott Alinity ci. The Alinity i Anti-HBs assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of antibody to hepatitis B surface antigen (anti-HBs) in human adult and pediatric serum and plasma (dipotassium EDTA, lithium heparin, andsodium heparin) and neonatal serum on the Alinity i analyzer. It is intended for quantitative measurement of antibody response following hepatitis B virus (HBV) vaccination, determination of HBV immune status, and for the laboratory diagnosis of HBV disease associated with HBV infection when used in conjunction with other laboratory results and clinical information. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Alinity ci. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of anti-HBs in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined and incubated. The anti-HBs present in the sample binds to the rHBsAg coated microparticles. The mixture is washed. Recombinant HBsAg acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-HBs in the sample and the RLUs detected by the system optics. The concentration of anti-HBs in the specimen is determined using an active Alinity i Anti-HBs calibration curve.For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.  |
| **Clinical Significance** | The Alinity i Anti-HBs assay determines the concentration of anti- HBs present in human serum and plasma.HBV is a major cause of liver disease and is endemic worldwide. The virus can be transmitted through direct contact with blood and body fluids including sexual contact. The incubation period for HBV infection can range from 1 to 6 months averaging around 6 to 8 weeks. Typical acute clinical symptoms of HBV hepatitis include malaise, jaundice, gastroenteritis, and fever. However, HBV infection can also result in subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. Although most adult patients with HBV infection completely recover from acute illness and clear the virus, 5 to 10% of patients with HBV may become chronic carriers.It is estimated that over 300 million people worldwide are chronic carriers of the virus. Chronic HBV infection is associated with the development of hepatocellular carcinoma. In HBV infected neonates, approximately 90% develop chronic hepatitis B infection.Anti-HBs assays are often used to determine the success of hepatitis B vaccination. The presence of anti-HBs has been shown to be important in protection against HBV infection. Numerous studies have demonstrated the effectiveness of the hepatitis B vaccine to stimulate the immune system to produce anti-HBs and to prevent HBV infection.Assays for anti-HBs are also used to monitor the convalescence and recovery of hepatitis B infected individuals. The presence of anti- HBs after acute HBV infection and loss of hepatitis B virus surface antigen (HBsAg) can be a useful indicator of disease resolution. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV or HBV vaccination. |
| **Instrument** | **PRIMARY METHOD**: Abbott Alinity ciBackup Method:Mayo Medical Laboratories (HBAB) |
| **Sunquest Test Code** | **HBSAB** |
| **Specimen** | **Preferred Sample type:** Serum/SST.Also acceptable: Lithium Heparin, Sodium Heparin and EDTA (not acceptable for Mayo Medical Laboratories send out testing.)**Sample Draw Volume:** 2.1 mL**Minimum Processed Sample Volume:** 200 µL of serum or plasma**Stability:** 3 days at room temperature, 7 days at 2-8°C, 2 years at -20°C or colder. **Transport:** Ship refrigerated at 2-8°C to Minneapolis lab**Rejection criteria:** Unlabeled specimens, incorrect sample type• Maximum number of replicates sampled from the same sample cup: 10Priority Loaded:Sample volume for first test: 125 μLSample volume for each additional test from same sample cup: 75 μLLoaded Routinely:Sample volume for first test: 150 μLSample volume for each additional test from same sample cup: 75 μL**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.–– **Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.**• After mixing, place reagent cartridges in an upright position for 2 hours before use to allow bubbles that may have formed to dissipate.• If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.• Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity i Anti-HBs Reagent | 07P8851 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date. Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 2 hours before use. May be used immediately after removal from 2-8°C storage.**On-board:** 30 Days |
| Abbott Alinity i Anti-HBs Calibrator | 07P8801 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.  |
| Anti-HBs Specimen Diluent | 07P8841 | **Store at**: 2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, Store tightly capped. Return to refrigerated storage after use. |

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| **Risk and Safety:** | Contains sodium azide. Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Recap and dispose of in appropriate Hazardous Waste Container unless empty, in which case reagent bottles can be disposed of in regular trash. |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 3.31 – 1000.0 mIU/mL |
| Reference Material: | Anti-HBs Calibrators |
| Suggested Calibration Levels | A – 0.0 mIU/mLB – 10.0 mIU/mLC – 50.0 mIU/mLD – 100.0 mIU/mLE – 500.0 mIU/mLF – 1000.0 mIU/mL |
| Verification Scheme: | n=6 |
| Verification Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| AMR | Verification of AMR is accomplished with each calibration.* Cal Verification and AMR verification are performed at least once every six (6) months.
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| **Quality Control** | Bio-Rad Viroclear Control, and Bio-Rad Virotrol II (2)**Frequency:** Run both controls each day of use.**Stability:** 60 Days at 2-8°C. Store Upright.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Limitations of the Procedure** | * + For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
	+ A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus.
	+ Results obtained with the Alinity i Anti-HBs assay may not be used interchangeably with values obtained with different manufacturers’ assay methods.
	+ Results from immunosuppressed patients should be interpreted with caution.
	+ Assay does not differentiate between vaccines and natural infections.
	+ Performance characteristics have not been established for therapeutic monitoring.
	+ A reactive anti-HBs result does not exclude co-infection by another hepatitis virus.
	+ The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages.
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| **Reference Range** |

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| **Numerical Value** | **Interpretation** |
| <8.0 mIU/mL | Non-Reactive |
| 8.0 – 12.0 mIU/mL | Equivocal |
| >12.01 mIU/mL | Reactive |

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| **Critical Values** | None specified |
| **Dilutions** |

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| Max Auto Dilution: | 1:25 |
| Maximum Manual Dilution: | 1:100 with Anti-HBs Specimen Diluent.It is recommended that dilutions not exceed 1:100.Add 10 μL of the sample to 990 μL of Alinity i Anti-HBs SpecimenDiluent (07P8841). |
| Diluent: | Onboard Diluent |
| Instrument Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 3.31, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Interpretation and Result Reporting** | Each result will cross the interface as the first test result. The results that will cross over in OEM will be Reactive, Nonreactive or Equivocal and the numerical value. Each result will have the comment “Check procedure for repeat and interpretation protocol.” Use the Alinity i Anti-HBs Interpretation chart (pictured below on page 7) to determine if a retest is required. If repeat testing is not needed (i.e. result is nonreactive), manually accept the Sunquest result. **If retesting is required** :1. Take specimen off the analyzer and check for clots, red cells, or other particulate matter. **Recentrifuge if any are seen or suspected.**
2. Manually order the specimen **in duplicate** with an ‘R’ in front of the accession number to signify the accession number that crosses OEM is a retest. For example, accession number “H111” would be manually ordered on the Alinity as “RH111”. Dilute samples 1:25 using automated dilution and 1:100 manual dilution as necessary.
3. When testing is complete, both results will cross into Sunquest in two different cups. To accept results in Sunquest, you will have to manually modify the correct accession number without the (R) in OEM. Sunquest will ask CHANGE EXISTING ACCESSION NUMBER (Y/<N>). Type Y then press ENTER and enter the accession number you wish to report.
4. **Go to the analyzer and check results**
5. If both repeat tests are <8 (non reactive), then manually accept the Sunquest result of nonreactive.
6. If both of the repeat tests are ≥8 to <12 (equivocal), then manually accept the Sunquest result of equivocal.
7. If one of the repeat tests are <8 **or** >12 and the other test is ≥8 or < 12 equivocal, then you must (M) modify the result in Sunquest. When modifying results, you must enter the equivocal **numeric result** from the analyzer. Sunquest will append an equivocal comment. **Do not free-text results or the correct interpretation will not append**.
8. If both repeat tests are >12 (reactive), then accept the Sunquest result of reactive once dilutions are complete.
9. Results will automatically dilute by instrument 1:25 dilution. Samples >25,000 should be diluted 1:100 using Anti-HBs Specimen Diluent as listed in the Dilutions section of this document.
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| **Result Reporting (Continued)** | **Alinity i Anti-HBs Interpretation**

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| **Initial Result** | **Retest Result** | **Result** | **Interpretation** | **What to enter into Sunquest** |
| <8.0 mIU/mL (Nonreactive) | No Retest Required | Nonreactive | “Individual is considered not immune to HBV infection.” | Accept Numerical Result and Nonreactive |
| ≥8.0 mIU/mL to < 12.0 mIU/mL (Equivocal) | Both of the duplicate retests are <8.0 mIU/mL | Nonreactive | “Individual is considered not immune to HBV infection.” | Accept Numerical Result and Nonreactive |
| One or both of the duplicate retest results are ≥8.0 mIU/mL to < 12.0 mIU/mL | Equivocal | “The immune status of the individual should be further assessed by considering other factors, such as clinical status, follow-up testing, associated risk factors, and the use of additional diagnostic information.” | Modify the Result and **enter the Numerical Value for the Equivocal result** from the Architect **(Should be ≥8.0-<12)** |
| Both of the duplicate retests are >12.0 mIU/mL | Reactive | “Individual is considered immune to HBV infection.” | Accept Numerical Result and Reactive |
| ≥12.0 mIU/mL (Reactive) | No Retest Required if below 1000.0. Results >1000.0 should be diluted with the 1:25 automated instrument dilution. Results >25,000 should be manually diluted 1:100 using the Anti-HBs Specimen Diluent  | Reactive | “Individual is considered immune to HBV infection.” | Accept Numerical Result and Reactive |

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| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity i Anti-HBs reagent cartridge insert sheet Abbott Laboratories, Abbott Park, IL, 60064. Revised Date June 2019.
2. Abbott Alinity i Anti-HBs calibrator insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised March 2019.
3. Abbott Alinity i Anti-HBs Specimen Diluent insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised March 2019.
4. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised July 30, 2015.
5. Bio-Rad Viroclear Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 July 2017.
6. Bio-Rad Virotrol II Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 October 2017.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos | May 15, 2018 | New Procedure |
| 2 | Erin Bartos | October 28, 2018 | Noted quantitative result and interpretation are reported. |
| 3 | Erin Bartos | May 1, 2019 | Updated QC for URT |
| 4 | Erin Bartos | October 28, 2020 | Changed from Architect to Alinity. |

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