| **Hepatitis B Surface Antigen Qualitative (HBsAg)** |
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| **Purpose** | This procedure provides instructions for performing HEPATITIS B SURFACE ANTIGEN QUALITATIVE II on the Abbott Alinity i. The Alinity i HBsAg Qualitative II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric serum and plasma and neonate serum on the Alinity i analyzer. The assay may also be used to screen for HBV infection in pregnant women to identify neonates who are at risk for acquiring hepatitis B during the perinatal period. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with the hepatitis B virus (HBV) (state of infection or associated disease not determined) in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis B infection. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Alinity ci.  |
| **Principle** | This assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. (Note: Ancillary Wash Buffer is added in a second incubation step, so the assay file performs a two-step assay protocol).Sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture and incubated. The HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridiniumlabeled conjugate. 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 HBsAg in the sample and the RLUs detected by the system optics. The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | The causative agent of serum hepatitis is HBV which is an enveloped DNA virus. During infection, HBV produces an excess of HBsAg, also known as Australia antigen, which can be detected in the blood of infected individuals. It is responsible for binding the virus to the liver cell and is the target structure of neutralizing antibodies. HBsAg is the first serological marker after infection with HBV, appearing 1 to 10 weeks after exposure and 2 to 8 weeks before the onset of clinical symptoms. HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within 6 months indicates a chronic HBsAg carrier state. HBsAg assays are used to identify persons infected with HBV and to monitor the status of infected individuals in combination with other hepatitis B serological markers. In most countries, testing for HBsAgis part of the antenatal screening program to identify HBV infected mothers and to prevent perinatal HBV infection by subsequent immunization.Specimens nonreactive by Alinity i HBsAg Qualitative II are considered negative for HBsAg. A reactive specimen must be retested in duplicate by Alinity i HBsAg Qualitative II to determinewhether it is repeatedly reactive. Specimens found to be repeatedly reactive by the Alinity i HBsAg Qualitative II assay should be sent out for a confirmatory assay. If the specimen is neutralized, the specimen is considered confirmed positive for HBsAg. It is recommended that confirmatory testing be performed before disclosing HBsAg status. |
| **Instrument** | **PRIMARY METHOD:** Abbott Alinity ciBackup Method: Mayo Medical Laboratories |
| **Sunquest Test Code** | **HBSA** |
| **Specimen** | **Preferred Sample type:** Serum/SSTOther sample types are acceptable but cannot be used for confirmatory testing. See package insert for more details. **Preferred Sample Draw Volume**: 3.6 mL blood**Minimum Processed Sample Volume:** 1.0 mL of serum **Stability:** 24 hours at room temperature, 6 days at 2-8°C, 2 years at -20°C or colder. Avoid more than 3 freeze/thaw cycles. **Transport:**  Send samples to Minneapolis lab refrigerated at 2-8°C. Reactive reflexed samples should be sent frozen to MML.**Rejection criteria:** Unlabeled specimens, incorrect sample type**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or 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** | **Reagent Handling:**• 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 1 hour 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 mayadversely affect results.

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity i HBsAg Qualitative II Reagent Kit | 08P1021 | **Store at:** 2 – 8 °C**Unopened:** 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 1 hour before use.May be used immediately after removal from 2-8°C storage.**Opened:** 30 Days. 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. May be used immediately after removal from 2-8°C storage.**On-board:** 30 Days.  |
| Abbott Alinity i HBsAg Qualitative II Calibrator Kit | 08P1002 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, Store tightly capped with new replacement cap. Store in an upright position. Return to refrigerated storage after use.stable until expiration date when stored and handled as directed. The analyzer will track In-use Stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer. The analyzer will not allow the use of the calibrator if the In-use Stability has been exceeded. Maximum In-use Stability can be found in the Assay Parameter Report. For additional information on calibrator Inuse Stability, refer to the Alinity ci-series Operations Manual, Section 5. |

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| **Risk and Safety:** | Contains methylisothiazolones. May cause an allergic skin reaction. Avoid breathing mist, vapors, and spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves, protective clothing, and eye protection. Dispose of in proper waste container; when reagent is empty, this may be disposed of in regular trash. |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 0-10.0 IU/mL |
| Reference Material: | HBsAg Qualitative II Calibrators |
| Suggested Calibration Levels | CAL 1 – 0.5244 IU/mLCAL 2 – 0 IU/mL |
| Verification Scheme: | n=2 |
| 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 not required for qualitative tests. |
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| **Quality Control** | Bio-Rad Viroclear and Bio-Rad Virotrol I**Frequency:** Both levels for each day of use.**Stability:** 60 Days at 2-8°C.**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](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) 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.
* As this is a qualitative test, Viroclear should be nonreactive and Virotrol II should be reactive. Do not report results unless both results meet these criteria.
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| **Limitations of the Procedure** | * Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits that employ mouse monoclonal antibodies.
* Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
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| **Limitations of the Procedure (Cont.)** | * The effectiveness of the Alinity i HBsAg Qualitative assay for use in screening blood, , or tissue donors has not been established.
* Assay performance characteristics have not been established when the Alinity i HBsAg Qualitative assay is used in conjunction with other manufacturers’ assays for specific HBV markers.
* Current methods for the detection of hepatitis B surface antigen may not detect all potentially infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with hepatitis B virus. A nonreactive test result in individuals with prior exposure to hepatitis B may be due to antigen levels below the detection limit of this assay or lack of antigen reactivity to the antibodies in this assay.
* If the Alinity i HBsAg Qualitative results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
* For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
* Results obtained with the Alinity i HBsAg Qualitative assay may not be used interchangeably with values obtained with different manufacturers’ assay methods.
* A reactive HBsAg result does not exclude co-infection by another hepatitis virus.
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| **Reference Range** |

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| **Numerical Value** | **Interpretation** |
| <1.0 IU/mL | Negative |

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| **Critical Values** | None specified |
| **Dilutions** | Do not dilute. |
| **Result Reporting** | Results will cross the interface into OEM for Alinity method code MACI. Each result will have the comment “Check procedure for repeat and interpretation protocol.” Use the Alinity i HBsAg Interpretation chart below to determine if repeat testing is required. If a repeat test 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 necessary.**
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 Architect as “RH111” with two replicates.
3. When testing is complete, both results will cross into Sunquest in two different cups.
4. To accept results in Sunquest, you will have to manually retype the correct accession number without the (R) for the result you wish to report in OEM. Sunquest will ask CHANGE EXISTING ACCESSION NUMBER (Y/<N>). Type Y then press ENTER to enter the accession number you wish to report.
5. **\*Go to the analyzer and check results**\*
6. If both repeat tests are <1.00 (non reactive), then manually accept one of the Sunquest results of nonreactive.
7. If both or one of the repeat tests are ≥1.00, accept the result of presumptive positive. Confirmatory testing will reflex to Mayo Medical Laboratories. Mayo Medical Laboratories test HBAG will automatically reflex for confirmation and a label will print. Place label on sample and place in the Send outs freezer for transport to MML. For samples that are QNS to send to MML, call the patient location/provider immediately for redraw.

**Alinity i Initial HBsAg Results**

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| **Initial Result (S/CO)** | **Instrument Interpretation** | **Retest Procedure** |
| <1.00 | Nonreactive | No Retest Required |
| ≥ 1.00 | Reactive | Retest in Duplicate |

**Alinity i Repeat Test HBsAg Results**

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| **Instrument Interpretation** | **Interpretation Appended Comment** | **What to Result in Sunquest** |
| Both repeat test results are nonreactive. (Both S/CO values are <1.00) | “Specimen considered negative for HBsAg” | Accept the result of nonreactive. |
| One or both results are reactive. (Both S/CO values are ≥ 1.00) | “Specimen repeatedly reactive, confirmatory testing reflexed.” Freeze a minimum of 0.5 mL to send to MML for reflexed HBAG testing. | Accept the result of Presumptive Positive OR type the NUMERICAL value from the Alinity.  |

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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 HBsAg Qualitative II reagent cartridge insert sheet Abbott Laboratories, Abbott Park, IL, 60064. Revised Date January 2020
2. Abbott Alinity i HBsAg Qualitative II calibrator insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised November January 2020.
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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 | May 1, 2019 | Updated QC for URT |
| 3 | Erin Bartos | October 28, 2020 | Changed to Alinity from Architect |

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