| **Hepatitis B Surface Antigen Qualitative (HBsAg)** |
| --- |
| **Purpose** | This procedure provides instructions for performing HEPATITIS B SURFACE ANTIGEN (Qualitative) on the Abbott Architect i1000SR. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | The ARCHITECT HBsAg Qualitative assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum using CMIA technology, with flexible assay protocols, referred to as Chemiflex. (Note: Ancillary Wash Buffer is added in a second incubation step so the assay files perform a two-step assay.) In the ARCHITECT HBsAg Qualitative assay, sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i* System optics. The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal 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. |
| **Clinical Significance** | The causative agent of serum hepatitis is hepatitis B virus (HBV) which is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (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 one to ten weeks after exposure and two to eight 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 six 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 HBsAg is part of the antenatal screening program to identify HBV infected mothers and to prevent perinatal HBV infection by subsequent immunization. Specimens nonreactive by ARCHITECT HBsAg Qualitative are considered negative for HBsAg. A reactive specimen must be retested in duplicate by ARCHITECT HBsAg Qualitative to determine whether it is repeatedly reactive. It is recommended that confirmatory testing be performed before disclosing HBsAg status. |
| **Instrument** | **PRIMARY METHOD:** Abbott Architect i1000SRBackup Method: Mayo Medical Laboratories |
| **Sunquest Test Code** | **HBSA** |
| **Specimen** | **Preferred Sample type:** Serum/SST **Preferred Sample Draw Volume**: 3.6 mL blood**Minimum Processed Sample Volume:** 1.0 mL of serum **Stability:** 24 hours at room temperature, 7 days at 2-8°C, 2 years at -20°C or colder. **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. Serum specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. **For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.** Serum specimensfrom patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
2. Serum should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Lipemic samples should be ultrafuged.
4. Transfer serum to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
 |
| **Reagents** |

|  |  |  |
| --- | --- | --- |
| ***Product Description*** | ***Product Code*** | ***Stability*** |
| HBsAg Reagent | 4P53 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer expiration date**Opened:** 30 Days**On-board:** 15 Days |
| HBsAg Calibrator | 4P53-01 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed.  |
| Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer |
| Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer |
| Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer |
| Reaction Vessels  | 07C15 (-02 or -03) | N/A |

 |
| **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 |

|  |  |
| --- | --- |
| Analytical Measuring Range: | 0-1.0 IU/mL |
| Reference Material: | HBsAg Calibrator 4P53-01 |
| Suggested Calibration Levels | 1 – 0.5244 IU/mL2 – 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
 |
| AMR | Verification of AMR is accomplished with each calibration.* Cal Verification and AMR verification are performed at least once every six (6) months.
 |
|  |

 |
| **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.**Sunquest Control names:** C-VIROC (Viroclear), C-VIRO1 (Virotrol I)**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until the cause of deviation has been identified and corrected
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
* When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
* 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.
 |
| **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.
 |
| **Limitations of the Procedure (Cont.)** | * The effectiveness of the ARCHITECT HBsAg Qualitative assay for use in screening blood, , or tissue donors has not been established.
* Assay performance characteristics have not been established when the ARCHITECT 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 ARCHITECT 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 ARCHITECT 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.
 |
| **Reference Range** |

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

 |
| **Critical Values** | None specified |
| **Dilutions** | Do not dilute. |
| **Result Reporting** | Results will cross the interface into OEM for Architect method code AI1. Each result will have the comment “Check procedure for repeat and interpretation protocol.” Use the ARCHITECT 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.

**Architect Initial HBsAg Results**

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

**Architect Repeat Test HBsAg Results**

|  |  |  |
| --- | --- | --- |
| **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 Architect.  |

 |
| **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 Architect reagent cartridge insert sheet Abbott Laboratories, Abbott Park, IL, 60064. Revised Date January 2016
2. Abbott Architect calibrator insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised November 2015.
3. Abbott Architect Package insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised January 2013
4. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised 2016-04-09.
5. Bio-Rad Lyphochek Specialty Immunoassay Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 January 2018
 |
| **Historical Record** |

|  |  |  |  |
| --- | --- | --- | --- |
| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos | May 15, 2018 | New Procedure |
|  |  |  |  |
|  |  |  |  |

 |