**CHEM.CENTAUR.ASSAY.10.0 HEPATITIS B SURFACE ANTIGEN BY ADVIA CENTAUR (HBSII) (HBSAGII)**

**PRINCIPLE**

The ADVIA Centaur HBsAG assay is a sandwich immunoassay using direct, chemiluminometric technology. Non-magnetic latex particles are added from the ancillary well. The Lite Reagent, packaged in a ReadyPack ancillary reagent pack, contains a biotinylated anti-HBs mouse monoclonal capture antibody and an acridinium-ester labeled anti-HBs mouse monoclonal antibody. HBsAg in the sample complexes with the antibodies and streptavidin-coated magnetic latex particles in the Solid Phase capture the HBsAg antibody complexes.

The sample is incubated simultaneously with Solid Phase, Lite Reagent, and Ancillary Reagent. Antibody-antigen complexes will form if hepatitis B surface antigen is present in the sample.

The system automatically performs the following steps:

1. Dispenses 100 µL of sample into a cuvette
2. Dispenses 60 µL of Ancillary Pack Reagent and incubates for 5 minutes at 37°C.
3. Dispenses 105 µL of Solid Phase reagent and 40 µL of Lite Reagent and incubates the mixture for 18 minutes at 37°C.
4. Separates the Solid Phase from the mixture and aspirates the unbound reagent
5. Washes the cuvette with Wash 1
6. Dispenses 300 µL each of Acid Reagent, and Base Reagent to initiate the chemiluminescent reaction
7. Reports results according to the selected option, as described in the system operating instructions or in the online help system

The relative light units (RLUs) detected by the ADVIA Centaur System are used to calculate the Index Value from the Master Curve. A result of reactive or non-reactive is determined according to a cutoff of 1.0 Index Value established with the calibrators.

**DOCUMENT OWNER**

Manager, Chemistry & Special Chemistry

**RELATED DOCUMENTS**

CHEM.CENTAUR.ASSAY.10.1 Reporting HBSAGII

CHEM.CENTAUR.ASSAY.10.2 Hepatitis Codes

CHEM.CENTAUR.ASSAY.10.3 Hepatitis Stocking

CHEM.CENTAUR.ASSAY.10.4 Result Code Interpretation for Hepatitis

CHEM.CENTAUR.ASSAY.10.5 Reporting Summary for Hepatitis

CHEM.CENTAUR.ASSAY.10.6 Hepatitis Information

CHEM.CENTARU.ASSAY.10.7 Verifying Centaur Hepatitis Results

**SPECIMEN**

1. Specimen Requirements

1. Serum

2. Plasma (Potassium EDTA, Lithium or Sodium heparin)

B. Stability

1. Refrigerated (2-8°C)

a. Serum / Plasma (Lithium Heparin)

1). Pour off tube – 24hr @ room temp (18-35C)

2). Pour off tube – 14d @ 2-8C

2. Frozen (≤-20°C) – no more than 4 freeze/thaw cycles

C. Minimum volume – 100 µL

Note: This volume does not include the unusable volume in the sample container or the additional volume required when performing other tests on the same sample.

D. Special Instructions

1. Centrifuge within 24 hours of collection and pour off into clean vial for best results.

2. Test samples as soon as possible after collecting.

3. Store samples at 2-8°C if not tested within 8 hours.

4. Before placing samples on the Centaur, ensure that:

a. Samples are free of fibrin or other particulate matter.

b. Samples are free of bubbles.

c. Samples are free from obvious microbial contamination.

**REAGENTS**

A. Store the reagents upright at 2-8°C.

B. Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, Handling Reagents, in the ADVIA Centaur Assay Manual.

c. Protect from sunlight. Protect reagent packs from all light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2-8°C away from light.

| **Reagent Pack** | **Reagent** | **Volume** | **Ingredients** | **Storage** | **Stability** |
| --- | --- | --- | --- | --- | --- |
| ADVIA Centaur HBsAgII ReadyPack primary reagent pack | Solid Phase | 21.0 mL/ reagent pack | Streptavidin-coated magnetic latex particles in buffer with bovine serum albumin, bovine gamma globulin, goat serum, surfactant, sodium azide  (< 0.1%) and preservatives | 2-8°C | Until the expiration date on the pack label. Onboard stability – 60 days. |
| ADVIA Centaur HBsAgII Ready Pack primary reagent pack | Lite Reagent | 8.0 mL/ reagent pack | Acridinium ester-labeled monoclonal mouse anti-HBsAg ( ~6.0 ug/mL) in buffer with bovine serum, albumin, bovine gamma globulin, goat serum, mouse IgG, surfactant, sodium azide (<0.1%) and preservatives | 2-8C | Until the expiration date on the pack label. Onboard stability – 60 days. |
| ADVIA Centaur HBsAgII  ReadyPack  Ancillary  Reagent pack | Ancillary Reagent | 25.0 mL/ reagent pack | Biotinylated monoclonal mouse anti-HBsAg antibody (~ 2.0 µg/mL) and acridinium ester-labeled monoclonal mouse anti-HBsAg (~ 0.3 µg/mL) in buffer with bovine serum albumin, bovine gamma globulin, goat serum, mouse IgG, surfactant, sodium azide (< 0.1%) and preservatives | 2-8°C | Until the expiration date on the pack label. Onboard stability – 60 days. |
| HBsAgII Calibrator vials | Calibrators | 2.5 mL/ vial | High calibrator: purified human HBsAg in buffer with sodium azide  (< 0.1%) low calibrator: buffer with sodium azide (< 0.1%) | 2-8°C | Until the expiration date on the vial , or onboard 8 hours |
| ADVIA  Centaur  1  ANC  ReadyPack  Ancillary | Probe  Wash | 25.0 mL/  Reagent  Pack | 0.14 N sodium hydroxide | 2-8°C | Until the expiration date on the pack label or 14 consecutive days after accessing the ancillary reagent pack |
| ADVIA Centaur  1  WASH | Wash 1 | 1500 mL/pack | Phosphate buffered saline with sodium azide  (< 0.1%) and surfactant | 2-25°C | Until the expiration date on the vial or onboard 14 days |

**CALIBRATION**

HBsII Calibrator is provided in each kit. The calibrators are matched to the ReadyPack primary reagent pack. DO NOT mix calibrator lots with different lots of reagent packs.

1. The ADVIA Centaur HBsII assay requires a 2 point calibration:
   1. Every 21 days.
   2. When changing lot numbers of primary reagent packs.
   3. When replacing system components.
   4. When quality control results are repeatedly out of range (See QC Policy).
2. For Calibrator preparation and stability, see appropriate package insert.
3. For each new lot number of Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibrating values, and performing a calibration, refer to system operating instructions or to the online “HELP” system.

**PROCEDURE**

See procedure CHEM.CENTAUR.2.0, Advia Centaur Operating Procedure, for specific details.

**QUALITY CONTROL**

* + 1. Refer to QC Testing Intervals for appropriate control materials and testing intervals.
    2. Refer to QC Policy for criteria on accepting/rejecting patients based on control results.

**REPORTING RESULTS**

1. Reference Ranges: Non-reactive for HBsAg
2. Critical Values: None
3. The Cutoff Index Value of 1.00 is used to determine whether a sample is reactive or non-reactive for HBSAG.
   1. Samples with a calculated Index Value, value less than 1.00 are considered non-reactive (negative) for HBs, and should be reported as non-reactive (NREAC).
   2. Samples with initial results ≥ 1.0 Index Value require retest. Repeat the testing in duplicate.
      1. If 2 of the 3 results are < 1.00 Index Value report as NREAC.
      2. If 2 of the 3 results are still ≥ 1.00, do the confirmation test for HBSAG, report as RECCO (this will generate a confirmation test (HBSAG).
      3. Perform a confirmation.

**NOTE**: If only HBSAG is ordered (test 37567), it will be on worksheet CIMI9, result as NREAC or NEAC but do not do confirmation. (Don’t report as RECCO)

**NOTE**: Results >1000.0 Index also need to be confirmed.

**NOTE**: Mid America will send out the following specimens for testing with alternate methodology: Cord Blood, cadaver specimens, heat inactivated specimens, and body fluids other than serum or plasma such as saliva, urine, amniotic fluid or pleural fluid.

On all Hepatitis testing, there will be a disclaimer for neonates (<28 days of age) and adolescent (29 days to a8 years).

For Neonate (<28 days of age): The performance of assay has not been established with neonatal specimens.

For 29 days to 18 years: Assay characteristics have not been established for infants, children, or adolescent patients.

1. Computer Entry:
   1. Misys Entry:
   2. Function: MEM or OEM
   3. Worksheet: CI
   4. Method: CI4
   5. Test: HBsAg, HBAGX, HEPSC
   6. QLS Entry:
      1. QLS common pathway: 3, 3, 1
      2. Test code: 498/10306 37567 (HBSAG only – no confirmation)
      3. Worksheet: CIMI7 CIMI9 (for test 37567)
   7. Computer Downtime:

Refer to the Laboratory Computer Downtime procedure if LIS or HIS is non-functional.

* 1. HGsAg results are qualitative.

**PROCEDURE NOTES**

1. Clinical Significance

The ADVIA Centaur HBsAgII assay is a magnetic particle chemiluminometric immunoassay used to measure the amount of hepatitis B surface antigen in human serum and plasma. Hepatitis B virus (HBV) is endemic throughout the world and is the major cause of liver disease. HBV is transmitted through direct contact with blood and body fluids. Common modes of transmission include blood transfusion, needle puncture, direct contact with open wounds, sexual contact, and mother-neonate contact during birth.

The average incubation period for HBV infection is 6 to 8 weeks (range 1 to 6 months). Common clinical symptoms include malaise, fever, gastroenteritis, and icterus. HBV infection can result in typical icteric hepatitis, subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. In adults, 90 to 95% of patients with HBV infection completely recover from acute illness and clear the virus. Approximately 5 to 10% of patients with HBV become chronic carriers. In HBV infected neonates, approximately 90% develop chronic hepatitis B infection. It is estimated that over 300 million people worldwide are chronic carriers of the virus. Chronic HBV infection is clearly associated with the development of hepatocellular carcinoma.

1. Hepatitis B surface antigen (HBsAg) is a distinctive serological marker of acute or chronic hepatitis B infection. HBsAg is the first antigen to appear following infection with hepatitis B virus and is generally detected 1 to 10 weeks before the onset of clinical symptoms. HBsAg assays are routinely used to diagnose suspected HBV infection and to monitor the status of infected individuals to determine whether the infection has resolved or the patient has become a chronic carrier of the virus. In patients that recover from HBV infection, HBsAg levels disappear 3 to 5 months after the onset of the infection. In patients with chronic HBV infection, HBsAg levels may remain detectable for life. Prenatal HBsAg screening has been recommended so that newborns from HBV carrier mothers may obtain prophylactic treatment.
2. Back-up Method:

If testing cannot be performed, arrangements will be made to send testing to a referral lab. The referral lab will be determined by the manager, supervisor or lead technologist at the downtime.

**LIMITATIONS**

1. The ADVIA Centaur HBsAgII assay is limited to the detection of HBsAg in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma).
2. For diagnostic purposes, the ADVIA Centaur HBsAgII test results should always be assessed in conjunction with the patient’s medical history, clinical examination, and other findings.
3. It is recognized that the current methods for the detection of hepatitis B surface antigen may not detect 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.
4. The analytical sensitivity of the ADVIA Centaur HBsAgII assay was verified with WHO 1st International Reference Standard, 00/588 and the Boston Biomedica Inc. HBsAg sensitivity panel (ad and ay subtypes). The analytical sensitivity of the assay was determined to be 0.040 IU/mL and 0.034 PEI Units ad and 0.033 PEI Units ay at the 1.00 Index Cutoff. Refer to the *Analytical Sensitivity* section for additional information.
5. The performance of the ADVIA Centaur HBsAgII assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
6. The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients. Results from these individuals must be interpreted with caution.
7. 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 values may be observed. Additional information may be required for diagnosis.
8. INTERFERENCE

|  |  |
| --- | --- |
| **Serum specimens that are ….** | **Demonstrate ≤ 10% change in results up to ….** |
| hemolyzed | 500 mg/dL of hemoglobin |
| lipemic | 1000 mg/dL of triglycerides |
| icteric | 40 mg/dL of conjugated bilirubin |
| icteric | 40 mg/dL of unconjugated bilirubin |
| proteinemic (high) | 12 g/dL of protein |

For additional information on performance characteristics including cross reactivity, see the product information in the ADVIA Centaur ASSAY Manual.

**REFERENCES**

See ADVIA Centaur Assay Procedure, procedure for HBsII (10635153\_EN Rev. E, 2015-01).