Policy Statement	Core Laboratory personnel are responsible for insuring the specimen submitted for testing is acceptable and the procedure for performing the test is adhered to.
Purpose	This procedure provides technical instruction for the performance of HBsAG on the ADVIA Centaur System.
Scope	This procedure applies to testing Personnel authorized to perform testing on the ADVIA Centaur System. This group includes, but is not limited to Medical Laboratory Technicians/Technologists, as well as leads and supervisory personnel.
Responsibility	All above personnel are responsible for following the ADVIA Centaur System procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
Related Documents	CORE 6910 R ADVIA Centaur System Operation CORE 6950 R Infectious Disease Reportable Tests CORE 6035 R Daily Quality Control Procedure Daily Handling, Processing and Reporting Results

Title: Detection of Hepatitis B Surface Antigen in Serum on the ADVIA Centaur System

Reporting Results

Reference Interval

- Nonreactive (negative): less than 1.0 Index Value
- Reactive (positive): greater than or equal to 1.0 Index Value

Alert Values

N/A

Reporting Protocol for Alert Values

N/A

Units for Reporting Results

The system reports HBsAg results in Index Values and as reactive or nonreactive.

Reportable Range

The reportable range of the ADVIA Centaur HBsAg assay is 0.1 to 1000 Index Value.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The system reports HBsAg results in Index Values and as reactive or nonreactive.

- Samples with an Index Value of less than 1.0 are considered **nonreactive** (negative) for HBsAg.
- Samples with an Index Value of greater than or equal to 1.0 but less than or equal to 50 are considered **initially reactive** for HBsAg. Samples are repeated in duplicate.
- After repeat testing, if two of the three results are nonreactive (negative), the sample is considered **nonreactive** for HBsAg.
- After repeat testing, if at least **two of the three** results are preliminary reactive (positive), the samples is considered repeat reactive (positive) and the presence of HBsAg should be confirmed with a HBsAg confirmatory assay (HBSAGC). The confirmatory assay will be sent to a reference lab for testing.
- Samples with an Index Value of greater than 50, the specimen is **reactive** for HBsAg by ADVIA Centaur HBsAg assay.
- The cutoff for the ADVIA Centaur HBsAg assay was verified based on results of Receiver-Operator characteristics (ROC) Curve, the relative sensitivity and specificity generated from the clinical studies, and the seroconversion sensitivity.
- Sample results are invalid and must be repeated if the controls are out of range.

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

Procedure Notes

Dilutions

Do not dilute patient samples.

Reactive (Positive) Values

- The test must be repeated in duplicate.
- If two of the three results are nonreactive (negative), the sample is considered negative for HBsAg.
- If at least two of the three results are reactive (positive), the samples is repeatedly reactive (positive) and the presence of HBsAg should be confirmed with a HBsAg confirmatory assay.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Quality Control (QC)

QC Materials

Use ADVIA Centaur HBsAg quality control material to monitor assay performance.

QC Frequency

Analyze all levels of quality control material each shift that samples are analyzed.

Analyze all levels of quality control material each time a two-point calibration is performed.

Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the quality control results do not fall within defined ranges technologists are expected to follow procedure CORE 6035 R.

Specimen Collection and Handling

Specimen Collection

- Serum is the recommended sample type for this assay.
- Test samples as soon as possible after collecting.
- Do not use specimens with obvious microbial contamination.
- This assay requires 100 *u*L of sample for a single determination.
- Samples are processed by centrifugation, typically followed by physical separation of the serum from the red cells.
- Centrifugation may occur up to 24 hours post draw. When testing 10 samples where the centrifugation step was varied up to 24 hours post draw, no clinically significant differences were observed.
- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

Specimen Storage and Stability

- Keep tubes covered and upright at all times.
- Tightly cover and refrigerate specimens at 2 to 8°C if not tested immediately.

- Store specimens at 2 to 8°C up to 7 days.
- Store primary tube samples at 2 to 8°C up to 7 days.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage.
- Do not store in frost-free freezers.
- 10 samples tested after 4 freeze/thaw cycles showed no qualitative differences. Thoroughly mix thawed samples and centrifuge before using.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Samples maintained at room temperature up to 2 days or refrigerated up to 7 days demonstrated no qualitative differences. Store samples at 2 to 8C upon arrival. If shipment is expected to exceed 7 days, ship specimens frozen.

Reagents

Storage and Stability

- Store the reagents upright at 2–8 °C.
- Store Wash 1 upright at 2–25 °C.
- Primary and ancillary reagents stable until the expiration date on the pack label, or for 41 days onboard the system.
- Calibrators and controls stable until the expiration date on the vial label, or for 8 hours onboard the system.
- Wash stable until the expiration date on the pack label, or for 14 days onboard the system.

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Reagents Special Preparation

No special preparation of reagents is required.

Calibration

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

Two-point Calibration Interval

Use HBsAg Calibrator to perform two-point calibrations.

Perform a two-point calibration every 21 days. Additionally, calibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

Method Limitations

- The ADVIA Centaur HBsAg assay is limited to the detection of HBsAg in human serum.
- Assay performance characteristics have not been established when the ADVIA Centaur HBsAg assay is used in conjunction with other manufacturers' assay for specific HBV serological markers.
- Assay performance characteristics have not been established for testing of newborns.
- The performance of the ADVIA Centaur HBsAg assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum, such as saliva, urine, amniotic, or pleural fluids.
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.
- Do not use specimens with obvious microbial contamination.
- 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.

Principle of the Test

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:

- dispenses 100 *u*L of sample into a cuvette
- dispenses 120 *u*L of Lite Reagent and incubates for 5 minutes at 37C
- dispenses 105 *u*L of Solid Phase and 25 *u*L of Ancillary Reagent and incubates the mixture for 18 minutes at 37C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- dispenses 300 *u*L each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

A direct relationship exists between the amount of HBsAg activity present in the patient sample and the amount of relative light units (RLUs) detected by the system. A result of positive or negative is determined according to a cutoff of 1.0 Index Value established with the calibrators. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Clinical Application and Usefulness

The ADVIA Centaur HBsAg assay is an *in vitro* immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum using the ADVIA Centaur system. The assay may be used in conjunction with other serological and clinical information to diagnose individuals with acute or chronic hepatitis B infection. The assay may also be used to screen for hepatitis B infection in pregnant women to identify neonates who are at risk of acquiring hepatitis B during the perinatal period.

Equipment and Supplies

- ADVIA Centaur HBsAg Primary ReadyPack
- ADVIA Centaur HBsAg Ancillary ReadyPack
- ADVIA Centaur HBsAg Calibrator
- ADVIA Centaur HBsAg Quality Control Material
- ADVIA Centaur Wash 1
- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- ADVIA Centaur Cleaning Solution Concentrate
- ADVIA Centaur Acid Reagent (0.5% H_20_2, 0.1N HNO_3)

- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

References

1. Siemens HealthCare ADVIA Centaur HBsAg product insert, Revision J.
2. Siemens Diagnostics ADVIA Centaur Operator's Guide, Revision A
3. National Committee for Clinical Laboratory Standards (NCCLS). Clinical Laboratory Procedure Manuals—Third Edition (GP2-A3), 1996.

Technical Assistance

Siemens HealthCare Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number: 21251109