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 Anti-HBS 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 Operation

Title: Total Antibodies to Hepatitis B Surface Antigen (aHBs2) in Serum on the ADVIA Centaur XP System

Reporting Results

Reference Interval

- Nonreactive (negative): less than 8 mIU/mL
- Retest Zone: greater than or equal to 8 mIU/mL and less than or equal to 12 mIU/mL. Sample must be retested in duplicate. After retesting, if 3 results are available and 2 results are ≥10 mIU/mL, then the sample is considered to be reactive. If 3 results are available and 2 results are ≤10 mIU/mL, then the sample is considered to be nonreactive.
- Reactive (positive): greater than or equal to 12 mIU/mL

Alert Values

N/A

Reporting Protocol for Alert Values

N/A

Reportable Range

The reportable range of the Anti-HBs assay is 3.1 to 1000mIU/mL.

Interpretation of Results

The system reports anti-HBs antibody results in mIU/mL and as reactive (positive), nonreactive (negative), or as needing retest:

Nonreactive: Samples with an initial value <8 mIU/mL. Anti-HBs is below 10 mIU/mL and the patient is considered not to have protective immunity to HBV infection.

Reactive: Samples with an initial value ≥12 mIU/mL. Anti-HBs is detected at ≥10 mIU/mL and the patient is considered to have protective immunity to HBV infection.

Retest Zone: Samples with an initial value ≥8 and <12 mIU/mL. If results are within the retest zone after initial testing, samples must be retested in duplicate. After retesting, if 3 results are available and 2 results are ≥10 mIU/mL, then the sample is considered to be reactive. If 3 results are available and 2 results are <10 mIU/mL, then the sample is considered to be nonreactive.

Sample results are invalid and must be repeated if the controls are out of range.

The ADVIA Centaur Anti-HBs2 assay is traceable to the World Health Organization (WHO) Hepatitis B Immunoglobulin 1st International Reference Preparation (1977). Samples with a calculated value of
10 mIU/mL or greater are considered reactive (protective) in accordance with the CDC guidelines. The accepted criteria for immunity to HBV is anti-HBs activity ≥10 mIU/mL, as defined by the WHO International Reference Preparation.

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.

Procedure Notes

Dilutions

- The following information pertains to dilutions:
- Samples with anti-HBs levels greater than 1000 mIU/mL may be diluted and retested.
- Patient samples can be automatically diluted by the system or prepared manually.

- For automatic dilutions, ensure that ADVIA Centaur Multi-Diluent 11 is loaded and set the system parameters as follows:
    - Dilution point: ≤1000 mIU/mL
    - Dilution factor: 2, 5, 10
    - For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.
- Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
- Use Multi-Diluent 11 to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

High Dose Hook Effect

In the ADVIA Centaur Anti-HBs2 assay, patient samples with levels of antibodies to HBsAg as high as 200,000 mIU/mL do not demonstrate a decrease in the RLUs (high-dose hook effect). Specimens having anti-HBs activity greater than 200,000 mIU/mL are extremely rare.

Quality Control (QC)

QC Materials

Use aHBs 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.

Analyze all levels of quality control prior to use of a new reagent lot or new shipment of reagent.

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.
- Do not use specimens with obvious microbial contamination.
- This assay requires 100 *u*L of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates, dilutions, or other tests on the same sample.
- Centrifugation may occur up to 24 hours post draw.
- Before placing samples on the system, ensure that samples are free of fibrin or other particulate matter and that samples are free of bubbles. Remove any visual lipid layer.

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.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage.
- Do not store in frost-free freezers.

Reagents

Storage and Stability

- Store the reagents upright at 2–8 °C.
- Store Wash 1 upright at 2–25 °C.
- Primary reagents stable until the expiration date on the pack label, or for 28 days onboard the system.
- Lite Reagent, Solid Phase, and Ancillary Reagent are stable until the expiration date on the pack label or for 90 days onboard the system.
- Calibrators and controls stable until the expiration date on the vial label, or for 8 hours onboard the system.
- Wash 1 is stable until the expiration date on the pack label, or for 14 days onboard the system.

- Multi-Diluent 11 is stable at 2–8°C until the expiration date on the pack label or for 28 consecutive days after accessing the ancillary reagent pack.

CAUTION:

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

Calibration

Two-point Calibration Interval

Use the ADVIA Centaur Anti-HBs2 Calibrators provided with each kit to perform a two-point calibration for this method.

NOTE: The low and high calibrators provided in the ADVIA Centaur Anti-HBs2 kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

Perform a two-point calibration every 42 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

- Assay performance characteristics have not been established when the ADVIA Centaur Anti-HBs2 assay is used in conjunction with other manufacturers' assays for specific HBV serological markers; and therefore, users are responsible for establishing their own performance characteristics.
- Assay performance characteristics have not been established in pregnant women, or in populations of immunocompromised or immunosuppressed patients; and therefore, users are responsible for establishing their own performance characteristics in these populations.
- This assay does not differentiate between a vaccine-induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
- This assay is not intended for use in screening blood bank or plasma donors.
- The performance of the ADVIA Centaur Anti-HBs2 assay has not been established with cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.

- Do not use specimens with obvious microbial contamination.
- Results obtained with ADVIA Centaur Anti-HBs2 assay may not be used interchangeably with values obtained with different manufacturer assay methods.
- Individuals that have received blood component therapies, for example, whole blood, plasma, immunoglobulin administered during the previous 3–6 months may have a false reactive anti-HBs result due to passive transfer of anti-HBs.
- The prevalence of the analyte in the population tested will affect the assay's predictive value.
- A positive anti-HBs result does not exclude co-infection by another hepatitis virus.

Principle of the Test

The ADVIA Centaur Anti-HBs2 assay is a sandwich immunoassay using direct, chemiluminometric technology. HBsAg (ad and ay) are covalently coupled to magnetic latex particles in the Solid Phase. In the Lite Reagent, the HBsAg (ad and ay) is labeled with acridinium ester. Non-magnetic latex particles are added from the ancillary well.

The sample is incubated simultaneously with Lite Reagent, Solid Phase, and Ancillary Reagent. Antibody-antigen complexes will form if anti-HBs2 is present in the sample.

A direct relationship exists between the amount of anti-HBs activity present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Clinical Application and Usefulness

The ADVIA Centaur anti-HBs2 assay is an *in vitro* diagnostic immunoassay for the qualitative and quantitative determination of total antibodies to hepatitis B surface antigen in human adult, adolescent, and pediatric serum or plasma (EDTA, lithium-heparinized, or sodium heparinized) and neonatal samples using the ADVIA Centaur and ADVIA Centaur XP systems. The assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection in individuals prior to or following HBV vaccination or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is unknown.

This assay has not been FDA-cleared or approved for the screening of blood or plasma donors.

Equipment and Supplies

- ADVIA Centaur aHBs2 ReadyPack
- ADVIA Centaur Wash 1
- ADVIA Centaur Multi-Diluent 11 (optional)
- ADVIA Centaur Acid Reagent (0.5% H_20_2, 0.1N HNO_3)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- ADVIA Centaur aHBs2 Quality Control Material
- ADVIA Centaur Cleaning Solution Concentrate (~52.5 g/L sodium hypochlorite)
- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- Reagent Water

References

1. Siemens Healthcare Diagnostics ADVIA Centaur aHBs2 Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Centaur Reference Manual.
3. Siemens Healthcare Diagnostics ADVIA Centaur XP Operator's Guide.
4. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Approved Guideline, GP02-A5, 2006.
5. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry; Approved Guideline, EP07-A2, 2005.

Technical Assistance

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

Customer Service: 1-800-255-3232

Serial Number: 21251109