HBsAg (HBs)

Assay for the Detection of Hepatitis B Surface Antigen

Assay Summary

Sample Type	***Serum, potassium EDTA plasma, lithium or sodium heparinized plasma***
Sample Volume	***100 µL***
Calibrator	***HBsAg***

Contents

REF	*Contents*	*Number of Tests*
03393362	1 ReadyPack® primary reagent pack containing ADVIA Centaur® HBsAg Solid Phase and Ancillary Reagent 1 Ancillary Pack containing ADVIA Centaur HBsAg Lite Reagent LR ADVIA Centaur HBsAg Master Curve card ADVIA Centaur Conf Master Curve card 1 vial HBsAg Low Calibrator CAL L 1 vial HBsAg High Calibrator CAL H ADVIA Centaur HBsAg Calibrator Assigned Value card	200

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the Symbols,* in the ADVIA Centaur® Assay Manual.

Intended Use

The ADVIA Centaur HBsAg Assay is an *in vitro* diagnostic immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma (potassium EDTA, lithium or sodium heparinized) 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.

WARNING: United States federal law restricts this device to sale by or on the order of a physician.

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

Assay performance characteristics have not been established when the ADVIA Centaur HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Assay performance characteristics have not been established for testing of newborns, infants, or children.[1] The user is reponsible for establishing their own assay performance characteristics in these populations.

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur Instrument	
03395373	ADVIA Centaur Ancillary Probe Wash I [ANC]	2 ReadyPack ancillary reagent packs containing 25 mL per pack
03394660	ADVIA Centaur HBsAg quality control material	2 x 10.0 mL Negative Control [CONTROL] ⊖ 2 x 10.0 mL Positive Control [CONTROL] ⊕ Expected Value card
01137199 (112351)	ADVIA Centaur Wash I [WASH]	2 x 1500 mL/pack

Summary and Explanation of the Test

The ADVIA Centaur HBsAg 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.[2,3]

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.[2,3,4]

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.[2,3,5]

Assay 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:

- dispenses 100 µL of sample into a cuvette
- dispenses 120 µL of Lite Reagent and incubates for 5 minutes at 37°C
- dispenses 105 µL of Solid Phase and 25 µL of Ancillary Reagent and incubates the mixture for 18 minutes at 37°C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- dispenses 300 µL 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

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. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Specimen Collection and Handling

Serum, potassium EDTA plasma, and lithium or sodium heparinized plasma are the recommended sample types for this assay. Do not use specimens with obvious microbial contamination. 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 or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards,[6] and augmented with additional sample handling studies using the ADVIA Centaur HBsAg assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 24 hours post draw. When testing 10 serum samples where the centrifugation step was varied up to 24 hours post draw, no clinically significant differences were observed. Time to centrifugation was established for serum only. User must establish time to centrifugation for other matrices.
- Test samples as soon as possible after collecting. Samples may be stored at room temperature or on board instrument for up to 8 hours. If testing is not completed within 8 hours, samples may be stored at 2 to 8°C for up to 14 days.
- Store primary tube samples at 2 to 8°C up to 3 days (up to 12 hours for lithium Heparin). Keep samples stoppered and upright at all times. Primary tube samples include serum stored on the clot, plasma stored on packed red cells, and samples processed and stored in gel barrier blood collection tubes. When 10 samples in these primary tubes were tested up to 3 days, no clinically significant differences were observed.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Do not store in a frost-free freezer. When 10 samples were subject to 6 freeze/thaw cycles, no

clinically significant differences were observed. Thoroughly mix thawed samples and centrifuge at 10,000g for 2 minutes 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 8 hours or refrigerated up to 14 days demonstrated no qualitative differences. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 14 days, ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation (example: 1500xg for 10 minutes; follow tube manufacturer's recommendations).
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.

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.

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 sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur HBsAg ReadyPack primary reagent pack	Solid Phase	21.0 mL/ reagent pack	streptavidin-coated magnetic latex particles in buffer with bovine serum albumin, goat serum, surfactant, sodium azide (<0.1%) and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Ancillary Reagent	5.0 mL/ reagent pack	choline reagent (~69.8 mg/mL) and non-magnetic latex particles in buffer with sodium azide (<0.1%)	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
ADVIA Centaur HBsAg LR ReadyPack ancillary reagent pack	Lite Reagent	24.0 mL/ reagent pack	biotinylated monoclonal mouse anti-HBsAg antibody† (~1.0 µg/ mL) and acridinium ester-labeled monoclonal mouse anti-HBsAg† (~0.1 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. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
HBsAg calibrator vials	Calibrators	3.0 mL/ vial	high calibrator: purified human HBsAg in buffer with sodium azide (< 0.1%) low calibrator: recalcified normal human plasma with sodium azide (< 0.1%)	2-8°C	until the expiration date on the vial or onboard 8 hours

HBsAg quality control material vials*	Controls	10.0 mL/ vial	recalcified human plasma negative and positive for HBsAg with preservatives	2-8°C	until the expiration date on the vial or onboard 8 hours
ADVIA Centaur [ANC] 1 ReadyPack ancillary reagent pack*	Probe Wash	25.0 mL/ reagent pack	0.4 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 [WASH] 1	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

† Directed to the "*a*" region determinant

*See *Materials Required But Not Provided*

Precautions and Warnings

For *In Vitro* Diagnostic Use.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[7-9]

The negative control and low calibrator have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control and high calibrator contain human plasma that is reactive for HBsAg and has been assayed by FDA approved methods and found nonreactive for antibodies to HCV and antibody to HIV-1/2. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

Loading Reagents

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: 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.

Load the ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ReadyPack ancillary reagent packs in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Lite Reagent in the ancillary reagent pack provided in this kit is matched to the Solid Phase and Ancillary Reagent in the primary reagent pack. Do not mix Lite Reagent lots with different lots of Solid Phase and Ancillary Reagent.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	21 days

Additionally, the ADVIA Centaur HBsAg assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

CAUTION:

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

Master Curve Calibration

The ADVIA Centaur HBsAg assay requires a Master Curve calibration when using a new lot number of Solid Phase, Ancillary Reagent, and Lite Reagent. For each new lot number of Solid Phase, Ancillary Reagent, and Lite Reagent, 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 calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur HBsAg assay, use the ADVIA Centaur HBsAg Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur HBsAg Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HBsAg assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.

2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator vial is approximately 50 µL.

3. Gently mix the Low and High Calibrators and dispense at least 6 to 7 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur HBsAg assay, use ADVIA Centaur HBsAg quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HBsAg quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HBsAg assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. If the assay is re-calibrated after the beginning of a run, the QC must be rerun before running patient samples.Treat all quality control samples the same as patient samples

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 6 to 7 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the Expected Values, then do the following:
- consider the sample results invalid.
- investigate and determine the cause of the unacceptable control result.
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance
- when the condition is corrected, retest the controls and confirm that the results are within acceptable limits.
- it is advisable to repeat all of the patient specimens before reporting results for this run.

Sample Volume

This assay requires 100 µL 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 or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.
3. Place the racks in the entry queue.
4. Ensure that the assay reagents are loaded.
5. Start the entry queue, if required.

Procedural Notes

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.

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.

- The initial cutoff value was determined by testing 1500 HBsAg negative samples and commercially available HBsAg sensitivity and seroconversion panels. The cutoff for the ADVIA Centaur HBsAg assay was verified based on results generated from the clinical studies and the seroconversion sensitivity.
- The cutoff value (50.00 Index Value) for not performing confirmatory testing was determined by testing initially reactive HBsAg samples from known positive and negative sample populations in the ADVIA Centaur HBsAg Confirmatory method. The 50.00 cutoff for the ADVIA Centaur HBsAg Confirmatory assay was verified based on results generated from the clinical studies where 100% (95% CL 97.02 - 100%) of 129 known positive specimens with ADVIA Centaur HBsAg Index values greater than 50.00 were confirmed positive for the presence of HBsAg .
- Samples with an Index Value of less than 1.00 are considered nonreactive (negative) for HBsAg.
- Samples with an Index Value of greater than or equal to 1.00 but less than or equal to 50 are considered initially reactive for HBsAg. Perform repeat testing in duplicate and /or supplemental testing on these samples.
- **Heparin anticoagulants have been shown to reduce the Index values in some samples near the assay cut off. High negative results (0.70 – 0.99 Index) obtained on samples collected with lithium or sodium heparin anticoagulant should be interpreted accordingly. Additional testing is required including other HBV markers and supplemental tests.**
- After repeat testing, if two of the three results are nonreactive (negative), the sample is considered negative for HBsAg.
- After repeat testing, if at least two of the three results are reactive , the sample is considered repeat reactive for the presence of HBsAg. If a repeatedly reactive result is confirmed by supplemental tests, such as the ADVIA Centaur HBsAg Confirmatory assay the sample is positive for HBsAg.
- If the sample is greater than 50, the specimen is positive for HBsAg by the ADVIA Centaur HBsAg assay.

  NOTE: When the ADVIA Centaur HBsAg assay is used as a stand alone assay (for example in pregnant women being screened to identify neonates who are at risk for acquiring HBV during the perinatal period), all results > 1.00 should be considered initially reactive. Repeat testing and supplemental tests, such as the ADVIA Centaur HBsAg Confirmatory assay must be used to confirm the result.
- The magnitude of the ADVIA Centaur HBsAg assay result does not correlate to a quantitative amount of HBsAg present in the sample.

Caution: It has been reported that certain assays will not detect all HBV mutants.[10,11,12] If acute or chronic HBV infection is suspected and the HBsAg result is nonreactive it is recommended that other HBV serological markers be tested to confirm the HBsAg nonreactivity.

Limitations

- The ADVIA Centaur HBsAg assay is limited to the detection of HBsAg in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma).
- For diagnostic purposes, the ADVIA Centaur HBsAg test results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.
- It is recognized that the 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.
- The analytical sensitivity of the ADVIA Centaur HBsAg assay was verified with WHO 1st International Reference Standard, 80/549-1 and the Boston Biomedica Inc. HBsAg sensitivity panel (ad and ay subtypes). The analytical sensitivity of the assay was determined to be 0.066 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.
- 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 or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
- 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.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[13] 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.

Expected Results

The prospective study population for the ADVIA Centaur HBsAg assay consisted of 2740 patients. Of these 2740 patients, 965 patients (35.22%) were from the population considered at risk for hepatitis (high risk) due to lifestyle, behavior, occupation, or known exposure events, 847 patients (30.91%) were from the signs and symptoms population, 212 patients (7.74%) were from the dialysis population, and 716 patients (26.13%) were from the prenatal population. The prenatal population included serum samples from healthy, pregnant women in their first trimester (149 of 716 patients [20.81%]), second trimester (272 of 716 patients [37.99%]), or third trimester (295 of 716 patients [41.20%]) of pregnancy. The prospective study population was 38.43% Hispanic, 31.79% Caucasian, 22.63% Black, 2.85% Asian, and 4.31% from unknown or other ethnicity. The majority of patients were female (60.11% female and 39.89% male). The mean age was 41.0 years (range of 12 to 82 years). Patients in the prospective study population were from the following geographic regions: Florida (37.77%), Texas (33.80%), New York (22.23%), and California (6.20%).

The ADVIA Centaur HBsAg results for the prospective population for all sites combined by age group and gender are summarized in the following table:

Distribution of High Risk, Signs and Symptoms, Dialysis, and Prenatal Population by Age Group and Gender (All Testing Sites)

Age Range (Years)	Gender	Positive [a] (N)	(%)	Nonreactive [b] (N)	(%)	Total (N)	(%)
0–9	Male	0	—	0	—	0	—
	Female	0	—	0	—	0	—
	Overall	0	—	0	—	0	—
10–19	Male	1	14.29	6	85.71	7	6.60
	Female	0		99	100	99	93.40
	Overall	1	0.94	105	99.06	106	100
20–29	Male	6	7.14	78	92.86	84	14.74
	Female	7	1.44	479	98.56	486	85.26
	Overall	13	2.28	557	97.72	570	100
30–39	Male	18	9.18	178	90.82	196	32.67
	Female	16	3.96	388	96.04	404	67.33
	Overall	34	5.66	566	94.33	600	100
40–49	Male	35	9.09	350	90.91	385	54.46
	Female	8	2.48	314	97.52	322	45.54
	Overall	43	6.08	664	93.91	707	100
50–59	Male	29	10.06	259	89.94	288	58.18
	Female	5	2.41	202	97.59	207	41.82
	Overall	34	6.86	461	93.13	495	100
60–69	Male	9	10.34	78	89.66	87	46.52
	Female	1	1.00	99	99	100	53.48
	Overall	10	5.35	177	94.65	187	100
≥ 70	Male	4	8.89	41	91.11	45	61.64
	Female	0	—	28	100	28	38.36
	Overall	4	5.48	69	94.52	73	100
Unknown	Male	0	—	1	100	1	50
	Female	0	—	1	100	1	50
	Overall	0	—	2	100	2	100
Total	Male	102	9.33	991	90.67	1093	39.89
	Female	37	2.24	1610	97.76	1647	60.11
	Overall	139	5.07	2601	94.93	2740	100

a Samples with an Index Value ≥ 1.00 were confirmed positive

b Samples with an Index Value < 1.00 and initially reactive samples that did not confirm positive

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[14]

Performance Characteristics

Results by Specimen Classification

The HBV disease classification for each patient in the high risk, signs and symptoms, and dialysis populations (2024 patients total) was determined by serological assessment using resultant hepatitis marker profiles obtained from results of commercially available, FDA-approved reference assays. The serological assessment included the following 6 HBV markers: hepatitis B virus surface antigen (HBsAg), hepatitis B virus e antigen (HBeAg), total antibody to hepatitis B virus core antigen (anti-HBc Total), IgM antibody to hepatitis B core antigen (anti-HBc IgM), total antibody to HBeAg (anti-HBe), and total antibody to hepatitis B virus surface antigen (anti-HBs) (quantitative). Testing of these specimens occurred at each study site. The individual ADVIA Centaur HBV assay result was compared to the reference HBV assay result and to the patient classification. No patients were excluded from the complete study set due to incomplete reference HBV serological results.

Each patient's HBV infection status was classified based on a single specimen and the reactive (+)/nonreactive(-) patterns of the 6 HBV reference serological markers. Disease classification for each patient was based only on the HBV serological marker results, and was not affected by additional laboratory or clinical information. There were 31 unique reference marker patterns observed using the ADVIA Centaur HBsAg assay. These patterns are presented in the following table:

Classification by Reference Markers (All Testing Sites)

	HBV Reference Markers					
HBV Classification	***HBsAg***	***HBeAg***	***IgM Anti-HBc***	***Total Anti-HBc***	***Anti-HBe***	***Anti-HBs (>10 mIU/mL)***
Acute	+	+	+	+	+	–
Acute	+	+	+	+	–	–
Acute	+	–	+	+	+	–
Chronic	+	+	–	+	+	–
Chronic	+	+	–	+	–	+
Chronic	+	+	–	+	–	–
Chronic	+	–	–	+	+	+
Chronic	+	–	–	+	+	–
Chronic	+	–	–	+	–	+
Chronic	+	–	–	+	–	–
Chronic	+	+	+	+	–	+
Early Recovery	–	–	+	+	+	+
Early Recovery	–	–	+	+	+	–
Early Recovery	–	–	+	+	–	+
Early Recovery	–	–	+	+	–	–
Early Recovery	–	–	–	+	+	–
Recovery	–	–	–	+	+	+
Recovery	–	–	–	–	+	+
Recovered	–	–	–	+	–	+
Recovered	–	–	–	+	–	–
HBV Vaccine	–	–	–	–	–	+

Response						
Not previously infected	–	–	–	–	–	–
Uninterpretable	+	–	–	–	–	+
Uninterpretable	+	–	–	–	–	–
Uninterpretable	–	+	–	–	–	+
Uninterpretable	–	+	–	–	–	–
Uninterpretable	–	–	+	–	–	–
Uninterpretable	–	–	–	–	+	–
Uninterpretable	–	+	–	+	–	+
Uninterpretable	–	+	–	+	–	–
Uninterpretable	–	+	–	+	+	+

+ = Reactive

– = Nonreactive

Comparison of Results

2024 samples in the high risk, signs and symptoms, and dialysis populations were run using the ADVIA Centaur HBsAg assay and a reference HBsAg assay for each HBV specimen classification. The following results were obtained:

Comparison of Results in High Risk, Signs and Symptoms, and Dialysis Populations by HBV Classification
ADVIA Centaur HBsAg Assay versus HBsAg Reference Assay (All Testing Sites)

	Reference HBsAg Negative		*Reference HBsAg Positive*		*Total* [a]
	ADVIA Centaur HBsAg Assay		*ADVIA Centaur HBsAg Assay*		
HBV Classification	*Positive* *N*	*Nonreactive* *N*	*Positive* *N*	*Nonreactive* *N*	*N*
Acute	0	0	10	1	11
Chronic	0	0	109	2	111
Early Recovery	0	126	0	0	126
Recovery	1	210	0	0	211
Recovered	3	320	0	0	323
HBV Vaccine Response	0	384	0	0	384
Not Previously Infected	2	826	0	0	828
Uninterpretable	1	22	3	4	30
Total	7	1888	122	7	2024

a Total number of test results by HBV categories

Percent Agreement

The agreement between the ADVIA Centaur HBsAg assay and a reference HBsAg assay for each HBV specimen classification is listed in the following table:

Percent Agreement and Confidence Intervals by HBV Classification in High Risk, Signs and Symptoms, and Dialysis Populations
ADVIA Centaur HBsAg Assay versus HBsAg Reference Assay (All Testing Sites)

HBV Classification	Positive Percent Agreement % (x/n) [a]	95% Exact Confidence Interval	Negative Percent Agreement % (x/n) [b]	95% Exact Confidence Interval
Acute	90.91 (10/11)	58.72 to 99.77	--	--
Chronic	98.20(109/111)	93.64 to 99.78	--	--
Early Recovery	--	--	100.00 (126/126)	97.11 to 100.00
Recovery	--	--	99.53 (210/211)	97.39 to 99.99
Recovered	--	--	99.07 (320/323)	97.31 to 99.81
HBV Vaccine Response	--	--	100.00 (384/384)	99.04 to 100.00
Not Previously Infected	--	--	99.76 (826/828)	99.13 to 99.97
Uninterpretable	42.86 (3/7)	9.90 to 81.59	95.65 (22/23)	78.05 to 99.89
Overall	94.57 (122/129)	89.14 to 97.79	99.63 (1888/1895)	99.24 to 99.85

a *x = the number of ADVIA Centaur HBsAg results that are confirmed positive in agreement with the reference HBsAg confirmed positive results; n = the total number of reference HBsAg results that are confirmed positive*

b *x = the number of ADVIA Centaur HBsAg results that are nonreactive in agreement with the reference HBsAg; n = the total number of reference HBsAg results that are nonreactive*

Comparison of Results: Retrospective Population

A population of commercially sourced HBV acute and HBV chronic samples was also tested using both the ADVIA Centaur HBsAg assay and a reference HBsAg assay in single replicates. The following results were obtained.

Comparison of Results in Retrospective HBV Infected Population
ADVIA Centaur HBsAg Assay versus Reference HBsAg Assay (All Testing Sites)

	Reference HBsAg Assay Negative		Reference HBsAg Assay Positive		
HBV Classification [a]	ADVIA Centaur HBsAg Assay		ADVIA Centaur HBsAg Assay		
	Positive (N)	Nonreactive (N)	Positive (N)	Nonreactive (N)	Total (N) [b]
Acute	0	0	49	0	49
Chronic	0	0	102	2	104
Total	0	0	151	2	153

a Vendor assignment of acute specimens was based on positive HBsAg and Anti-HBcM test results. Assignment of chronic specimens was based on positive HBsAg test result 6 months after diagnosis of HBV. Vendor assignment was verified based on single replicate testing of HBsAg, Anti-HBcM, Anti-HBc Total, and Anti-HBs assays at sites.

b Total number of test results by HBV categories.

Percent Agreement

The agreement between the ADVIA Centaur HBsAg assay and the reference HBsAg assay for the retrospective acute and chronic HBV infected population is summarized by testing site in the following table:

Percent Agreement and Confidence Intervals by Testing Sites in Retrospective HBV Population ADVIA Centaur HBsAg Assay versus Reference HBsAg Assay

Testing Site	Population	Positive Percent Agreement % (x/n) [a]	95% Exact Confidence Interval (CI)	Negative Percent Agreement % (x/n) [b]	95% Exact Confidence Interval (CI)
Florida	Acute	100 (14/14)	76.84 – 100	—	—
	Chronic	94.29 (33/35)	80.84 – 99.30	—	—
	Overall	95.92 (47/49)	86.02 – 99.50	—	—
Texas	Acute	100 (35/35)	90 – 100	—	—
	Chronic	100 (35/35)	90 – 100	—	—
	Overall	100 (70/70)	94.87 – 100	—	—
New York	Acute	—	—	—	—
	Chronic	100 (34/34)	88.72 – 100	—	—
	Overall	100 (34/34)	88.72 – 100	—	—
All Sites	Acute	100 (49/49)	92.75 – 100	—	—
	Chronic	98.08 (102/104)	93.23 – 99.77	—	—
	Overall	98.69 (151/153)	95.36 – 99.84	—	—

a x = the number of ADVIA Centaur HBsAg results that are positive in agreement with the reference HBsAg positive results; n = the total number of reference HBsAg results that are positive

b x = the number of ADVIA Centaur HBsAg results that are nonreactive in agreement with the reference HBsAg results; n = the total number of reference HBsAg results that are nonreactive

Prospective Study in Pregnant Women

Serum samples were prospectively collected from a U.S. prenatal population including 716 healthy, pregnant women who were in the first, second, or third trimester of pregnancy. By testing these samples from pregnant women using the ADVIA Centaur HBsAg assay and the reference HBsAg assay, the performance of the ADVIA Centaur HBsAg assay in identifying neonates who were at risk for HBV infection during the perinatal period was evaluated.

Results of HBsAg testing (reactive and nonreactive) were compared using the ADVIA Centaur HBsAg assay and the reference HBsAg assay for the prenatal population. A comparison of results for patients in their first, second, and third trimester for all testing sites combined is presented in the following table:

Comparison of Results in Prenatal Population
ADVIA Centaur HBsAg Assay versus Reference HBsAg Assay

HBV Classification	Reference HBsAg Assay Negative		Reference HBsAg Assay Positive		
	ADVIA Centaur HBsAg Assay		ADVIA Centaur HBsAg Assay		
	Positive (N)	Nonreactive (N)	Positive (N)	Nonreactive (N)	Total (N) [a]
1st Trimester	0	148	1	0	149
2nd Trimester	0	268	4	0	272
3rd Trimester	0	290	5	0	295
All	0	706	10	0	716

a Total number of test results by categories

Seroconversion Panels

Commercially available HBV patient seroconversion panels were tested using the ADVIA Centaur HBsAg assay to determine the seroconversion sensitivity of the assay. The performance of the ADVIA Centaur HBsAg assay on the seroconversion panels closely matched the performance of the reference assay. The following results were obtained:

Panel ID	HBsAg Positive Result From Initial Draw Date		Reference Assay vs ADVIA Centaur Assay
	Reference Assay (Days)	ADVIA Centaur Assay (Days)	Difference in Bleed Numbers [a]
PHM 907	51	51	0
PHM 912	42	42	0
PHM 919	19	19	0
PHM 926	12	9	+2
PHM 933	7	7	0
PHM 932	61	61	0

a The difference in bleed numbers is relative to the reference assay. For example, a +2 means that the reference assay required 2 additional bleeds before reactivity was determined as compared to the time-point when ADVIA Centaur assay confirmed positive.

Analytical Sensitivity

An HBsAg assay's ability to detect low levels of HBsAg is dependent on the analytical sensitivity of the assay and the assay's precision. To examine the analytical sensitivity of the ADVIA Centaur HBsAg three standard series with known levels of HBsAg were evaluated. The analytical sensitivity at the assay's cut off was determined by linear regression.

Series	Cutoff (Index = 1.00)	95% Exact Confidence Interval
WHO 1st IRP Standard, 80/549	0.066 IU/mL	0.0658 – 0.0662
Boston Biomedica Inc. HBsAg Sensitivity panel (ad subtype), PHA 806	0.034 PEI Units/mL	0.028 – 0.04
Boston Biomedica Inc. HBsAg Sensitivity panel (ay subtype), PHA 806	0.033 PEI Units/mL	0.032 – 0.034

Precision

Precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-A.[15] Samples were assayed in three replicates twice a day for 20 days. The following testing was performed at Bayer HealthCare on one ADVIA Centaur instrument. The following results were obtained:

	Mean Index	Within-run		Among run		Among Date		Total	
Sample		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
Positive Control	4.76	0.09	1.9	0.09	1.8	0.09	2.0	0.16	3.3
Serum 1	0.56	0.04	7.2	0.01	2.5	0.03	5.5	0.05	9.4
Serum 2	1.82	0.06	3.2	0.05	2.5	0.04	2.1	0.08	4.6
Serum 3	3.57	0.07	2.0	0.04	1.2	0.06	1.7	0.10	2.9
Serum 4	17.07	0.37	2.2	0.34	2.0	0.39	2.3	0.64	3.7
K2 EDTA	0.49	0.05	9.8	0.04	7.4	0.02	3.7	0.06	12.8
K2 EDTA	1.86	0.07	3.6	0.00	0.0	0.05	2.6	0.08	4.5
K2 EDTA	3.93	0.11	2.7	0.03	0.9	0.09	2.2	0.14	3.6
K2 EDTA	19.13	0.37	2.0	0.36	1.9	0.26	1.4	0.58	3.1
Lithium heparin	0.36	0.04	9.7	0.02	6.6	0.03	7.0	0.05	13.7
Lithium heparin	1.77	0.08	4.6	0.04	2.1	0.04	2.5	0.10	5.6
Lithium heparin	3.77	0.08	2.1	0.06	1.6	0.06	1.6	0.11	3.0
Lithium heparin	16.92	0.30	1.8	0.20	1.2	0.24	1.4	0.43	2.5
Sodium heparin	0.42	0.50	11.2	0.02	3.8	0.02	5.4	0.05	13.0
Sodium heparin	2.08	0.10	4.7	0.04	1.8	0.00	0.0	0.10	5.0
Sodium heparin	3.72	0.10	2.8	0.07	1.9	0.00	0.0	0.13	3.4
Sodium heparin	19.87	0.39	2.0	0.43	2.2	0.20	1.0	0.62	3.1

System Reproducibility

System reproducibility was determined by testing a five member panel and controls using three reagent lots, on three instruments at three sites over six days. Panel members were run in replicates of five on each day. The following results were obtained:

	Mean Index	Within Run		Between Run		Between Site		Between Lot		Total	
Sample		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.09	0.015	NA*	0.000	NA	0.001	NA	0.000	NA	0.015	NA
2	0.70	0.088	12.44	0.066	9.37	0.153	21.77	0.000	NA	0.188	26.77
3	1.75	0.096	5.49	0.076	4.36	0.160	9.13	0.074	4.25	0.215	12.27
4	4.29	0.155	3.62	0.124	2.90	0.232	5.41	0.226	5.28	0.380	8.87
5	19.96	0.482	2.42	0.415	2.08	0.726	3.64	0.861	4.31	1.294	6.48
Negative Control	0.13	0.055	NA	0.019	NA	0.059	NA	0.000	NA	0.083	NA
Positive Control	4.63	0.177	3.83	0.112	2.42	0.212	4.57	0.060	1.30	0.304	6.56

* NA = Not applicable

Cross-Reactivity

The specificity of the ADVIA Centaur HBsAg assay was evaluated by testing 205 distinct serum specimens from 22 potentially cross-reacting groups. Patient samples from the following groups were tested: HAV antibody, HCV antibody, VZV, CMV, EBV, HSV-1, Syphilis, Toxoplasma IgM, Toxoplasma IgG, Rubella, Non-viral Liver Disease, Human anti-mouse antibodies (HAMA), Systemic Lupus Erythematosus, Rheumatoid factor and Anti-nuclear antibody (ANA). Of these 22 sub-groups, 202 out of 205 specimens were observed to be non-reactive. One ANA specimen and 2 HIV+ specimens were reactive. The following table shows testing result summary:

Sample Group	Number Tested	Centaur HBsAg Assay non-reactive	Centaur HBsAg Assay reactive
Rheumatoid Factor	9	9	0
ANA - SLE	7	6	1[a]
Rubella IgG^+	10	10	0
Non Viral Liver Disease	10	10	0
HAV total	5	5	0
HCV IgG^+	10	10	0
VZV IgG^+	10	10	0
CMV IgG^+	10	10	0
HAMA	9	9	0
Flu vaccine recipient	10	10	0
Toxoplasma IgM	7	7	0
Toxoplasma IgG	8	8	0
EBV IgG^+	10	10	0
EBV IgM^+	10	10	0
Syphilis	10	10	0
HSV-1 IgG^+	10	10	0
HSV-1 IgM^+	10	10	0
HCV +	10	10	0
HIV +	10	8	2[b]
Rubella IgM+	10	10	0
CMV IgM+	10	10	0
VZV IgM+	10	10	0
Total Samples	205	202	3

a One sample was repeatedly reactive and not confirmed with less than 50% neutralization using the ADVIA Centaur HBsAg Confirmatory assay

b Two Samples were repeatedly reactive and confirmed positive for HBsAg with greater than 50% neutralization using the ADVIA Centaur HBsAg Confirmatory assay

In addition, the following bacterial and viral antigens were spiked into HBsAg negative and positive serum specimens: S. aureus, P. aeruginosa and E.coli, EBV, CMV, Rubella, VZV. The bacteria were spiked to 1000 and 10,000 CFU/mL. The viral antigens were spiked to 1 ug/mL and 1 ng/mL.

HBsAg results of various bacterial spikes

Spike Material	*Reactivity before spike*	*Reactivity after spike*
S. aureus 1000 CFU/mL	Non-reactive	Non-reactive
S. aureus 10,000 CFU/mL	Non-reactive	Non-reactive
P. aeruginosa 1000 CFU/mL	Non-reactive	Non-reactive
P. aeruginosa 10,000 CFU/mL	Non-reactive	Non-reactive
E. coli 1000 CFU/mL	Non-reactive	Non-reactive
E. coli 10,000 CFU/mL	Non-reactive	Non-reactive
S. aureus 1000 CFU/mL	Reactive	Reactive
S. aureus 10,000 CFU/mL	Reactive	Reactive
P. aeruginosa 1000 CFU/mL	Reactive	Reactive
P. aeruginosa 10,000 CFU/mL	Reactive	Reactive
E. coli 1000 CFU/mL	Reactive	Reactive
E. coli 10,000 CFU/mL	Reactive	Reactive

HBsAg results of various viral antigen spikes

Spike Material	*Reactivity before spike*	*Reactivity after spike*
EBV 1 µg/mL	Non-reactive	Non-reactive
EBV 1 ng/mL	Non-reactive	Non-reactive
CMV 1 µg/mL	Non-reactive	Non-reactive
CMV 1 ng/mL	Non-reactive	Non-reactive
Rubella 1 µg/mL	Non-reactive	Non-reactive
Rubella 1 ng/mL	Non-reactive	Non-reactive
VZV 1 µg/mL	Non-reactive	Non-reactive
VZV 1 ng/mL	Non-reactive	Non-reactive
EBV 1 µg/mL	Reactive	Reactive
EBV 1 ng/mL	Reactive	Reactive
CMV 1 µg/mL	Reactive	Reactive
CMV 1 ng/mL	Reactive	Reactive
Rubella 1 µg/mL	Reactive	Reactive
Rubella 1 ng/mL	Reactive	Reactive
VZV 1 µg/mL	Reactive	Reactive
VZV 1 ng/mL	Reactive	Reactive

Endogenous Interferents

The potentially interfering effects of conjugated bilirubin, unconjugated bilirubin, hemoglobin, triglycerides, hyper IgG and low protein were evaluated using 10 serum specimens following the guidelines described by NCCLS EP7-P [16] for interference due to endogenous substances. This study was performed with serum only and it is recommended that the user establish interference with other specimen types, i.e., anticoagulants.

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 total protein
proteinemic (low)	3 g/dL of total protein
hyper IgG	6 g/dL of immunoglobulin G

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. CDRH, Guidance for Industry and FDA Staff, Review Assessment of Pediatric Medical Devices, May 1, 2004.
2. Gitlin N. Hepatitis B: Diagnosis, Prevention, and Treatment. Clinical Chemistry 1997, 43:8(B):1500-1506.
3. Mahoney, FJ. Update on Diagnosis, Management, and Prevention of Hepatitis B Virus Infection. Clinical Microbiology Reviews 1999,12(2):351-366.
4. Juszczyk, J. Clinical Course and Consequences of Hepatitis B Infection. Vaccine 2000, 18:S23-S25.
5. Vivek R. Treatment of Hepatitis B. Clin Cornerstone 2001, 3(6):24-36.
6. National Committee for Clinical Laboratory Standards. Procedures for handling and processing of blood specimens; second edition; approved guideline. NCCLS Document H18-A2; Wayne (PA):NCCLS;1999 Oct. 40p.
7. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
8. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from occupationally acquired infections-second edition; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS; 2001 Dec. 105p.
9. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
10. Alexopoulou A, Baltayiannis G, et. al, Hepatitis B Surface Antigen Variant with Multiple Mutations in the *a* Determinant in an Agammaglobulinemic Patient. JCM 2004;42:2861-2865.
11. Jeantet D, Chemin I, et.al, Cloning and Expression of Surface Antigens from Occult Chronic Hepatitis B Virus Infections and Their Recognition by Commercial Detection Assays. J Gen Virol 2004;73:508-515.
12. Hou J, Wang Z, et.al, Prevalence of Naturally Occurring Surface Gene Varients of Hepatitis B Virus in Nonimmunized Surface Antigen-Negative Chinese Carriers. Hepatology 2001;34:1027-1034
13. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27–33.
14. National Committee for Clinical Laboratory Standards. How to define, determine, and utilize reference intervals in the clinical laboratory; approved guideline. NCCLS Document C28-A. Wayne (PA):NCCLS;1995.
15. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices–second edition; approved guideline. NCCLS Document EP5-A. Wayne (PA):NCCLS;1999.
16. National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; proposed guideline. NCCLS document EP7-P. Wayne (PA):NCCLS;1986.

HBsAg Confirmatory (Conf)

Assay for the Qualitative Confirmation of Hepatitis B Surface Antigen

Assay Summary

Sample Type	*Serum, potassium EDTA plasma, lithium or sodium heparinized plasma*
Sample Volume	*200 µL (2 x 100 µL)*
Calibrator	*HBsAg*

Contents

REF	*Contents*	*Number of Tests*
03393818	1 ReadyPack® ancillary reagent pack containing ADVIA Centaur® HBsAg Confirmatory Reagent A RGT A 1 ReadyPack ancillary reagent pack containing ADVIA Centaur HBsAg Confirmatory Reagent B RGT B	100

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the Symbols,* in the ADVIA Centaur® Assay Manual.

Intended Use

The ADVIA Centaur HBsAg Confirmatory Assay is an *in vitro* diagnostic immunoassay for the qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma (potassium EDTA, lithium or sodium heparin) using the ADVIA Centaur system. The assay is intended to be used to confirm the presence of HBsAg in samples that are repeatedly reactive using the ADVIA Centaur HBsAg Assay.

WARNING: United States federal law restricts this device to sale by or on the order of a physician.

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

Assay performance characteristics have not been established when the ADVIA Centaur HBsAg and HBsAg Confirmatory assays are used in conjunction with other manufacturers' assays for specific HBV serological markers.

Assay performance characteristics have not been established for testing of newborns, infants, or children.[1]

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur System	
03393362	ADVIA Centaur HBsAg Assay (200 tests)	1 ReadyPack primary reagent pack containing ADVIA Centaur HBsAg Solid Phase and Ancillary Reagent 1 Ancillary Pack containing ADVIA Centaur HBsAg Lite Reagent [LR] ADVIA Centaur HBsAg Master Curve card ADVIA Centaur HBsAg Confirmatory Master Curve card 1 vial HBsAg Low Calibrator [CAL] (L) 1 vial HBsAg High Calibrator [CAL] (H) ADVIA Centaur HBsAg Calibrator Assigned Value card
03394660	ADVIA Centaur HBsAg quality control material*	2 x 10.0 mL Positive Control [CONTROL] (+) 2 x 10.0 mL Negative Control [CONTROL] (−) Expected Value card
07948423 (110314)	ADVIA Centaur Multi-Diluent 2 [M-DIL]	2 x 10 mL/pack
03395373	ADVIA Centaur Ancillary Probe Wash 1 [ANC]	2 ReadyPack ancillary reagent packs containing 25 mL per pack
01137199 (112351)	ADVIA Centaur Wash 1 [WASH]	2 x 1500 mL/pack

* Only the Positive Control is run in the ADVIA Centaur HBsAg Confirmatory assay.

Summary and Explanation of the Test

The ADVIA Centaur HBsAg Confirmatory assay is based on the principle of specific antibody neutralization. The sample is incubated with polyclonal (human) antibody to HBsAg. The antibody will bind to HBsAg present in the sample thereby neutralizing the antigen. The neutralized HBsAg is blocked from binding to the antibodies in the assay. Blocking of the HBsAg by the assay antibodies results in a reduction in signal when compared to a second aliquot of sample that has been incubated with a non-neutralizing control reagent. The non-neutralizing control reagent serves as a control for the neutralization as well as the 0 percent baseline for calculation of the amount of reduction in signal as percent neutralization. A sample is considered positive for HBsAg if the percent neutralization is 50% or greater after treatment with the antibody (neutralizing reagent).

Assay Principle

The ADVIA Centaur HBsAg Confirmatory assay uses the principle of specific antibody neutralization to confirm the presence of HBsAg in a sample that is repeatedly reactive for HBsAg.

The sample is pretreated and tested in parallel; one sample aliquot is dispensed and incubated with a neutralizing reagent containing high titers of anti-HBs (Reagent A); the second sample aliquot is incubated with a non-neutralizing control reagent (Reagent B).

HBsAg in the patient sample is bound by the anti-HBs in Reagent A and not allowed to react

in the ADVIA Centaur HBsAg assay. When both aliquots are run in the ADVIA Centaur HBsAg assay, the inhibition of the RLU signal in the aliquot with Reagent A is compared to the RLU signal in the aliquot with Reagent B. The relative percent neutralization is calculated and an interpretation of the sample is generated.

The system automatically performs the following steps for sample pretreatment:

- dispenses 100 µL of sample into each of 2 cuvettes
- dispenses 50 µL of Reagent A in the first cuvette
- dispenses 50 µL of Reagent B in the second cuvette
- incubates for 29 minutes at 37°C

On the second pass of the ADVIA Centaur Confirmatory assay, the pretreated samples are tested following the ADVIA Centaur HBsAg assay principle. Refer to *Assay Principle* in the ADVIA Centaur HBsAg instructions for use.

Percent neutralization is calculated by comparing the RLU values obtained with Reagent A to those obtained with the control reagent, Reagent B. If the RLU value with Reagent B is below the cutoff, the assay is invalid. Refer to *Interpretation of Results* for a description of the assay interpretations and the calculation for percent neutralization.

Specimen Collection and Handling

Serum, potassium EDTA plasma, and lithium or sodium heparinized plasma, are the recommended sample types for this assay. Do not use specimens with obvious microbial contamination. 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 or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards,[2] and augmented with additional sample handling studies using the ADVIA Centaur HBsAg assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 24 hours post draw. When testing 10 serum samples where the centrifugation step was varied up to 24 hours post draw, no clinically significant differences were observed. Time to centrifugation was established for serum only. User must establish time to centrifugation for other matrices.
- Test samples as soon as possible after collecting. Samples may be stored at room temperature or on board system for up to 8 hours. If testing is not completed within 8 hours, samples may be stored at 2 to 8°C for up to 14 days.
- Store primary tube samples at 2 to 8°C up to 3 days (up to 12 hours for lithium heparin). Keep samples stoppered and upright at all times. Primary tube samples include serum stored on the clot, plasma stored on packed red cells, and samples processed and stored in gel barrier blood collection tubes. When 10 samples in these primary tubes were tested up to 3 days, no clinically significant differences were observed.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Do not store in a frost-free freezer. When 10 samples were subject to 6 freeze/thaw cycles, no clinically significant differences were observed. 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 8 hours or refrigerated up to 14 days demonstrated no qualitative differences. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 14 days, ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation.
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.

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.

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 sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur Conf RGT A ReadyPack ancillary reagent pack	Neutralizing Reagent	5.0 mL/ reagent pack	human plasma positive for antibodies to HBsAg with preservatives	2-8°C	until the expiration date on the pack label or 41 consecutive days after accessing the ancillary reagent pack
ADVIA Centaur Conf RGT B ReadyPack ancillary reagent pack	Non-Neutralizing Control Reagent	5.0 mL/ reagent pack	human plasma negative for HBsAg and antibodies to HBsAg with preservatives	2-8°C	until the expiration date on the pack label or 41 consecutive days after accessing the ancillary reagent pack
ADVIA Centaur M-DIL 2 ReadyPack ancillary reagent pack*	Multi-Diluent 2	10.0 mL/ reagent pack	goat serum with sodium azide (0.1%) and preservatives	2-8°C	until the expiration date on the pack label or 28 consecutive days after accessing the ancillary reagent pack
ADVIA Centaur APW 1 ReadyPack ancillary reagent pack*	Probe Wash	25.0 mL/ reagent pack	0.4 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 HBsAg ReadyPack primary reagent pack*	Solid Phase	21.0 mL/ reagent pack	streptavidin-coated magnetic latex particles in buffer with bovine serum albumin, goat serum, surfactant, sodium azide (<0.1%), and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Ancillary Reagent	5.0 mL/ reagent pack	choline reagent (~69.8 mg/ mL) and non-magnetic latex particles in buffer with sodium azide (<0.1%)	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*

ADVIA Centaur HBsAg [LR] ReadyPack ancillary reagent pack*	Lite Reagent	24.0 mL/ reagent pack	biotinylated monoclonal mouse anti-HBsAg antibody (~1.0 µg/mL) and acridinium ester-labeled monoclonal mouse anti-HBsAg (~0.1 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. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
HBsAg calibrator vials*	Calibrators	3.0 mL/ vial	high calibrator: purified human HBsAg in buffer with sodium azide (<0.1%) low calibrator: recalcified normal human plasma with sodium azide (<0.1%)	2-8°C	until the expiration date on the vial or onboard 8 hours
HBsAg quality control material vials*	Controls	10.0 mL/ vial	recalcified human plasma negative and positive for HBsAg with preservatives	2-8°C	until the expiration date on the vial or onboard 8 hours
ADVIA Centaur [WASH] 1**	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

* See *Materials Required But Not Provided.* Only the Positive Control is run in the ADVIA Centaur HBsAg Confirmatory assay.

** See *Materials Required But Not Provided.*

Precautions and Warnings

For *In Vitro* Diagnostic Use.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[3-5]

The human source components in the ADVIA Centaur HBsAg Confirmatory kit have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2.

Loading Reagents

Ensure that the system has sufficient primary reagent and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: 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.

NOTE: To perform the ADVIA Centaur HBsAg Confirmatory assay, load the ADVIA Centaur HBsAg ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ADVIA Centaur HBsAg Lite Reagent, ADVIA Centaur HBsAg Confirmatory Reagent A and Reagent B, ADVIA Centaur Multi-Diluent 2, and ADVIA Centaur Ancillary Probe Wash 1 ReadyPack ancillary reagent packs in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in the ADVIA Centaur HBsAg kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Lite Reagent ReadyPack ancillary reagent pack provided in the ADVIA Centaur HBsAg kit is matched to the Solid Phase and Ancillary Reagent. Do not mix Lite Reagent lots with different lots of Solid Phase and Ancillary Reagent.

Onboard Stability, Calibration Interval, and Master Curve Calibration

Because the ADVIA Centaur HBsAg Confirmatory assay uses ADVIA Centaur HBsAg primary reagent packs, refer to *Onboard Stability and Calibration Interval* and *Master Curve Calibration* in the ADVIA Centaur HBsAg instructions for use. The ADVIA Centaur HBsAg Confirmatory Master Curve card is included in the ADVIA Centaur HBsAg kit.

The ADVIA Centaur HBsAg Confirmatory Reagent A and Reagent B are stable onboard for 41 days with a calibration interval of 21 days.

Calibration

For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

The ADVIA Centaur HBsAg Confirmatory assay requires a valid ADVIA Centaur HBsAg assay calibration on the ADVIA Centaur System prior to performing the assay. Insure that both the ADVIA Centaur HBsAg and HBsAg Confirmatory calibrations have been completed.

Refer to *Calibration* in the ADVIA Centaur HBsAg instructions for use.

Quality Control

For quality control of the ADVIA Centaur HBsAg Confirmatory assay, use ADVIA Centaur HBsAg positive quality control material. The Positive Control should be run in the same manner as a repeat reactive sample.

For the run to be valid, the percent neutralization for the Positive Control must be greater than or equal to 50%, and a result of confirmed must be obtained.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HBsAg quality control barcode labels to identify the positive control sample cup when performing the ADVIA Centaur HBsAg Confirmatory assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance quality control samples must be assayed on each workshift that samples are analyzed. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure the positive control in duplicate.

1. Schedule the positive quality control sample to the worklist.
2. Label one sample cup with the quality control barcode label for the positive control.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control material and dispense at least 8 to 10 drops into the appropriate sample cup.
4. Load the sample cup in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control material remaining in the sample cup after 8 hours. Do not refill the sample cup when the contents are depleted; if required, dispense fresh quality control material.

Taking Corrective Action

If the quality control results are not within the ranges stated and do not yield ≥ 50% neutralization then do the following:

- consider the sample results invalid.
- investigate and determine the cause of the unacceptable control result.
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance
- when the condition is corrected, retest the controls and confirm that the results are within acceptable limits.
- it is advisable to repeat all of the patient specimens before reporting results for this run.

Sample Volume

This assay requires 200 µL 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 or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.
3. Place the racks in the entry queue.
4. Ensure that the assay reagents are loaded.
5. Start the entry queue, if required.

Procedural Notes

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.

Interpretation of Results

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

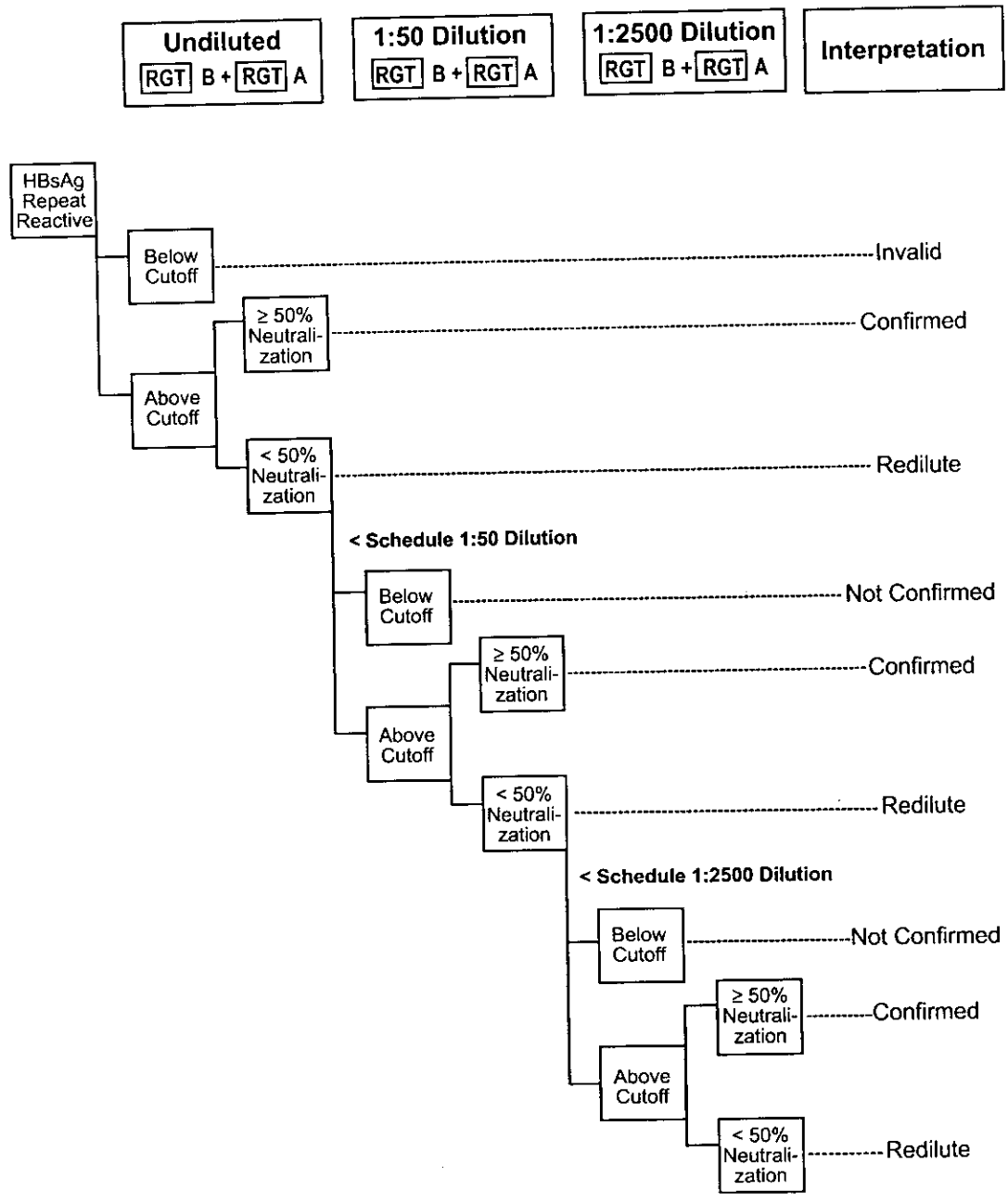
The system calculates the cutoff and percent neutralization automatically based on the calibration of the ADVIA Centaur HBsAg Confirmatory assay and the values obtained for the sample run with Reagent A and Reagent B.

NOTE: The cutoff is based on RLU values of the calibrators and not on the ADVIA Centaur HBsAg Index Value (Index Value = 1.0). This is so that the cutoff can be adjusted to allow for dilution of the sample with Reagent A and Reagent B.

The system reports ADVIA Centaur HBsAg Confirmatory results as Invalid, Redilute, Not Confirmed, or Confirmed.

- Samples are reported as Invalid if the sample run with Reagent B is below the cutoff value of the ADVIA Centaur HBsAg Confirmatory assay. The assay is invalid and should be repeated. If the interpretation is Invalid after repeat testing, it means a valid result cannot be obtained with this sample and a new sample should be obtained.
- Samples reported as Redilute require further dilution for confirmation.
- Samples reported as Not Confirmed are HBsAg negative.
- Samples reported as Confirmed are HBsAg positive.

Refer to the following testing algorithm for the interpretation of repeat reactive samples:


Undiluted
RGT B + RGT A
1:50 Dilution
RGT B + RGT A
1:2500 Dilution
RGT B + RGT A
Interpretation
HBsAg Repeat Reactive
Below Cutoff
Invalid
≥ 50% Neutrali-zation
Confirmed
Above Cutoff
< 50% Neutrali-zation
Redilute
< Schedule 1:50 Dilution
Below Cutoff
Not Confirmed
≥ 50% Neutrali-zation
Confirmed
Above Cutoff
< 50% Neutrali-zation
Redilute
< Schedule 1:2500 Dilution
Below Cutoff
Not Confirmed
≥ 50% Neutrali-zation
Confirmed
Above Cutoff
< 50% Neutrali-zation
Redilute

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[6] 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. Samples containing heterophilic antibodies should not neutralize using the ADVIA Centaur HBsAg Confirmatory assay.

For diagnostic purposes and to differentiate between acute and chronic HBV infection, the detection of HBsAg should be correlated with patient clinical information and other HBV serological markers. It is recognized that current methods for detection of hepatitis B surface antigen may not detect all potentially infected individuals. A false reactive HBsAg test result or invalid Confirmatory result does not exclude the possibility of exposure to or infection with hepatitis B.

Caution: It has been reported that certain assays will not detect all HBV mutants.[7,8,9] If acute or chronic HBV infection is suspected and the HBsAg result is nonreactive it is recommended that other HBV serological markers be tested to confirm the HBsAg nonreactivity.

Limitations

- The ADVIA Centaur HBsAg Confirmatory assay is limited to the confirmation of HBsAg in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma) in samples repeatedly reactive using the ADVIA Centaur HBsAg assay.
- The performance of the ADVIA Centaur HBsAg Confirmatory 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.
- 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.

Expected Results

The prospective study population for the ADVIA Centaur HBsAg assay and ADVIA Centaur HBsAg Confirmatory assay consisted of 2740 patients. Of these 2740 patients, 965 patients (35.22%) were from the population considered at risk for hepatitis (high risk) due to lifestyle, behavior, occupation, or known exposure events, 847 patients (30.91%) were from the signs and symptoms population, 212 patients (7.74%) were from the dialysis population, and 716 patients (26.13%) were from the prenatal population. The prenatal population included serum samples from healthy, pregnant women in their first trimester (149 of 716 patients [20.81%]), second trimester (272 of 716 patients [37.99%]), or third trimester (295 of 716 patients [41.20%]) of pregnancy. The prospective study population was 38.43% Hispanic, 31.79% Caucasian, 22.63% Black, 2.85% Asian, and 4.31% from unknown or other ethnicity. The majority of patients were female (60.11% female and 39.89% male). The mean age was 41.0 years (range of 12 to 82 years). Patients in the prospective study population were from the following geographic regions: Florida (37.77%), Texas (33.80%), New York (22.23%), and California (6.20%).

The ADVIA Centaur HBsAg results for the prospective population for all sites combined by age group and gender are summarized in the following table:

Distribution of High Risk, Signs and Symptoms, Dialysis, and Prenatal Population by Age Group and Gender (All Testing Sites)

Age Range (Years)	Gender	Positive [a] (N)	(%)	Nonreactive [b] (N)	(%)	Total (N)	(%)
0–9	Male	0	—	0	—	0	—
	Female	0	—	0	—	0	—
	Overall	0	—	0	—	0	—
10–19	Male	1	14.29	6	85.71	7	6.60
	Female	0	—	99	100	99	93.40
	Overall	1	0.94	105	99.06	106	100
20–29	Male	6	7.14	78	92.86	84	14.74
	Female	7	1.44	479	98.56	486	85.26
	Overall	13	2.28	557	97.72	570	100
30–39	Male	18	9.18	178	90.82	196	32.67
	Female	16	3.96	388	96.04	404	67.33
	Overall	34	5.66	566	94.33	600	100
40–49	Male	35	9.09	350	90.91	385	54.46
	Female	8	2.48	314	97.52	322	45.54
	Overall	43	6.08	664	93.91	707	100
50–59	Male	29	10.06	259	89.94	288	58.18
	Female	5	2.41	202	97.59	207	41.82
	Overall	34	6.86	461	93.13	495	100
60–69	Male	9	10.34	78	89.66	87	46.52
	Female	1	1.00	99	99	100	53.48
	Overall	10	5.35	177	94.65	187	100
≥ 70	Male	4	8.89	41	91.11	45	61.64
	Female	0	—	28	100	28	38.36
	Overall	4	5.48	69	94.52	73	100
Unknown	Male	0	—	1	100	1	50
	Female	0	—	1	100	1	50
	Overall	0	—	2	100	2	100
Total	Male	102	9.33	991	90.67	1093	39.89
	Female	37	2.24	1610	97.76	1647	60.11
	Overall	139	5.07	2601	94.93	2740	100

a Samples with an Index Value ≥ 1.00 that were confirmed positive

b Samples with an Index Value < 1.00 and initially reactive samples that did not confirm positive

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[10]

Performance Characteristics

Results of testing using the ADVIA Centaur HBsAg Confirmatory assay for all testing sites by HBV classification are presented for the high risk, signs and symptoms, and dialysis populations in the following table. Samples from 133 patients that were repeatedly reactive using the ADVIA Centaur HBsAg assay were tested for neutralization using the ADVIA Centaur HBsAg Confirmatory assay.

Confirmatory Results for Reactive Samples by HBV Classification in High Risk, Signs and Symptoms, and Dialysis Populations (All Testing Sites)

	ADVIA Centaur HBsAg Assay		*Reference EIA HBsAg Assay*	
HBV Classification	*Repeat Reactive N*	*Positive By Neutralization N(%)*	*Reference Assay percent agreement with ADVIA Centaur (%) [a](N/N) [b]*	*95% Exact Confidence Intervals*
Acute	10	10 (100)	100% (10/10)	69.2 – 100
Chronic	109	109 (100)	100% (109/109)	96.7 – 100
Early Recovery	0	0	NA	NA
Recovery	1	1 (100)	0% (0/1)	NA
Recovered	5	3 (60)	0% (0/3)	0.0 – 70.8
HBV Vaccine Response	0	0	NA	NA
Not Previously Infected	4	2 (50)	0% (0/2)	0.0 – 84.2
Uninterpretable	4	4 (100)	75.0% (3/4)	19.4 – 99.4
Total	133	129 (96.99)	94.57% (122/129)	89.1 – 97.8

a The percentage of Reference EIA HBsAg positive by neutralization samples which were also positive by neutralization in the ADVIA Centaur HBsAg assay.

b The number of samples which were positive by neutralization in the Reference EIA HBsAg assay over the number of samples which were positive by neutralization in the ADVIA Centaur HBsAg assay.

The prenatal population included serum samples from healthy, pregnant women in their first trimester (149 of 716 patients [20.81%]), second trimester (272 of 716 patients [37.99%]), or third trimester (295 of 716 patients [41.20%]) of pregnancy. Results for the prenatal population are presented by trimester showing 100% agreement for the 10 confirmed positive samples (95% Exact CI = 69.2 to 100) and 100% agreement with the 706 nonreactive samples (95% Exact CI = 99.5 to 100).

Comparison of Results in Prenatal Population
ADVIA Centaur HBsAg Assay versus Reference HBsAg Assay

	Reference HBsAg Assay Negative		*Reference HBsAg Assay Positive*		
Population	*ADVIA Centaur HBsAg Assay*		*ADVIA Centaur HBsAg Assay*		
	Positive (N)	*Nonreactive (N)*	*Positive (N)*	*Nonreactive (N)*	*Total (N) [a]*
1st Trimester	0	148	1	0	149
2nd Trimester	0	268	4	0	272
3rd Trimester	0	290	5	0	295
All	0	706	10 [b]	0	716

a Total number of test results by categories

b All 10 samples were confirmed positive with the reference confirmatory assay and Centaur HBsAg Confirmatory assay

The ranges of index values for 143 repeatedly reactive samples from the high risk, signs and symptoms, dialysis, and prenatal populations using the ADVIA Centaur HBsAg assay were compared to neutralization results using the ADVIA Centaur HBsAg Confirmatory assay. These data are summarized in the following table:

Distribution of ADVIA Centaur HBsAg and HBsAg Confirmatory Results

ADVIA Centaur HBsAg Assay Repeat Reactive Results		*ADVIA Centaur HBsAg Confirmatory Assay Confirmed*	
Index Range	*N*	*N (%)*	*95% Exact Confidence Intervals*
≥ 1.0 – < 50	14	10 (71.40)	41.9 – 91.6
≥ 50 – < 500	27	27 (100)	87.2 – 100
≥ 500	102	102 (100)	96.4 – 100
Total	143	139 (97.20)	93.0 – 99.2

For interfering substances and cross-reactivity results refer to the ADVIA Centaur HBsAg labeling.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. CDRH, Guidance for Industry and FDA Staff, Review Assessment of Pediatric Medical Devices, May 1, 2004.
2. National Committee for Clinical Laboratory Standards. Procedures for handling and processing of blood specimens; second edition; approved guideline. NCCLS Document H18-A2; Wayne (PA):NCCLS;1999 Oct. 40p.
3. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
4. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from occupationally acquired infections-second edition; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS; 2001 Dec. 105p.
5. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
6. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27–33.
7. Alexopoulou A, Baltayiannis G, et. al, Hepatitis B Surface Antigen Variant with Multiple Mutations in the *a* Determinant in an Agammaglobulinemic Patient. JCM 2004;42:2861-2865.
8. Jeantet D, Chemin I, et.al, Cloning and Expression of Surface Antigens from Occult Chronic Hepatitis B Virus Infections and Their Recognition by Commercial Detection Assays. J Gen Virol 2004;73:508-515.
9. Hou J, Wang Z, et.al, Prevalence of Naturally Occurring Surface Gene Varients of Hepatitis B Virus in Nonimmunized Surface Antigen-Negative Chinese Carriers. Hepatology 2001;34:1027-1034.
10. National Committee for Clinical Laboratory Standards. How to define, determine, and utilize reference intervals in the clinical laboratory; approved guideline. NCCLS Document C28-A. Wayne (PA):NCCLS;1995.

Bayer HealthCare

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HBsAg (HBs)

Contents

REF	Contents
03394660	2 vials of Negative Control CONTROL
	2 vials of Positive Control CONTROL
	Expected Values Card and barcode labels

00365090 Rev. A, 2005-03

Intended Use

For monitoring the performance of the HBsAg and HBsAg Confirmatory assays on the ADVIA Centaur® Systems. The performance of the HBsAg quality control material has not been established with any other HBsAg or HBsAg Confirmatory assays.

"**Warning**: United States federal law restricts this device to sale by or on the order of a physician."

Control Description

Volume	Ingredients	Storage	Stability
10.0 mL/vial	Recalcified human plasma negative and positive for HBsAg with preservatives	2–8°C	Until the expiration date on the vial label or onboard–8 hours

WARNINGS:

For *In Vitro* Diagnostic Use.

CAUTION! POTENTIAL BIOHAZARD: The controls contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[1-3] Use eye protection and gloves when handling this product; wash hands after handling.

The negative control has been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control contains human plasma that is reactive for HBsAg and has been assayed by FDA-approved methods and found nonreactive for antibodies to HCV and antibody to HIV-1/2. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

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.

The results obtained using the HBsAg quality control material depend on several factors. Erroneous results can occur from improper storage, inadequate mixing, or sample handling errors associated with system or assay procedures.

The controls are not calibrators and should not be used for assay calibration.

Preparing the Quality Control Material

Gently swirl and invert the vials to ensure homogeneity.

Using the Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the HBsAg quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HBsAg assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 6 to 7 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

CAUTION: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Do not return any quality control materials back into the vials after testing because evaporation and contamination can occur, which may affect results.

For quality control of the ADVIA Centaur HBsAg Confirmatory assay, use ADVIA Centaur HBsAg positive quality control material. The Positive Control should be run in the same manner as a repeatable reactive sample.

Reviewing, Editing, and Printing Results

For detailed information about reviewing, editing, and printing quality control results, refer to the system operating instructions or to the online help system.

Expected Results

Refer to the *Expected Values* card for the assigned values specific for the lot number of the HBsAg quality control material. For additional information, refer to the reagent instructions for use.

For HBsAg Confirmatory results to be valid, the percent neutralization for the Positive Control must be greater than or equal to 50% and a result of confirmed must be obtained.

The mean values established should fall within the range specified in *Expected Values*. Individual results may fall outside the range.

Taking Corrective Action

If the quality control results do not fall within the suggested *Expected Values*, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance

Limitations

Values have not been established for assays other than the ADVIA Centaur HBsAg Assay. If the user wishes to use this control material with other assays it is their responsibility to establish appropriate ranges.

Control performance has not been established for matrices other than serum and plasma.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens; Approved guideline-2nd Edition. NCCLS document H18-A2. Wayne (PA):NCCLS;1999.
2. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
3. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS;2001.