HBsAgII (HBsII)

Current revision and date	Rev. B, 2013-03	
Product Name	ADVIA Centaur® HBsAgII assay (200 tests)	REF 10492138
Materials Required but not Provided	ADVIA Centaur HBsAg quality control material	REF 03394660
	ADVIA Centaur Ancillary Probe Wash 1	REF 03395373
	ADVIA Centaur Wash 1 (2 x 1500 mL)	REF 01137199
	ADVIA Centaur Wash 1 (2 x 2500 mL)	REF 03773025
Specimen Type	Serum, plasma (EDTA, lithium-heparin, or sodium-heparin)	
Sample Volume	100 µL	
Assay Range	0.1–1000 Index	
Onboard Stability, Readypack	60 days	
Calibration Interval	21 days	

Intended Use

The ADVIA Centaur HBsAgII (HBsII) assay is an *in vitro* immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult, adolescent, and pediatric serum and plasma (EDTA, lithium-heparin, or sodium-heparin), and neonatal samples using the ADVIA Centaur and ADVIA Centaur XP systems. 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.

Summary and Explanation

The ADVIA Centaur HBsAgII assay is a magnetic particle chemiluminometric immunoassay used to measure the amount of hepatitis B surface antigen in human serum and plasma.

Hepatitis B virus (HBV) is endemic throughout the world and is the major cause of liver disease. HBV is transmitted through direct contact with blood and body fluids. Common modes of transmission include 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. HBV infection, particularly in cases of chronic 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 remain detectable for life. Prenatal HBsAg screening has been recommended so that newborns from HBV carrier mothers may obtain prophylactic treatment.[2,3,5]

Principle of the Procedure

The ADVIA Centaur HBsAgII assay is a sandwich immunoassay using direct, chemiluminometric technology.

The Ancillary Pack Reagent containing biotinylated anti-HBs mouse monoclonal capture antibodies and an acridinium-ester-labeled anti-HBs mouse monoclonal antibody is added to the sample. The HBsAg in the sample complexes with the antibodies. A second acridinium-ester-labeled anti-HBs mouse monoclonal antibody is added from the primary reagent pack. Streptavidin-coated magnetic latex particles in the Solid Phase capture the HBsAg-antibody complexes.

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

A direct relationship exists between the amount of HBsAg activity present in the patient sample and the amount of relative light units (RLUs) detected by the system. A result of reactive or nonreactive 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.

Reagents

Reagent	Description	Storage	Stability
ADVIA Centaur HBsAg II ReadyPack primary reagent pack; Solid Phase	21.0 mL/ reagent pack: streptavidin-coated magnetic latex particles (60 mg/dL) in buffer with bovine serum albumin, bovine gamma globulin, goat serum, surfactant, sodium azide (< 0.1%) and preservatives	Store the reagents upright at 2–8° C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability–60 days.
ADVIA Centaur HBsAg II ReadyPack primary reagent pack; Lite Reagent	8.0 mL/ reagent pack: acridinium ester-labeled monoclonal mouse anti-HBsAg (~0.6 μg/mL) in buffer with bovine serum albumin, bovin gamma globulin, goat serum, mouse IgG, surfactant, sodium azide (< 0.1%) and preservatives	Store the reagents upright at 2–8° C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability–60 days.
ADVIA Centaur HBsAg II ReadyPack ancillary reagent pack; Ancillary Pack Reagent	25.0 mL/ reagent pack: biotinylated monoclonal mouse anti-HBsAg antibodies (~2.0 μg/mL) and acridinium ester-labeled monoclonal mouse anti-HBsAg (~0.3 μg/mL) in buffer with bovine serum albumin, bovine gamma globulin, goat serum, mouse IgG, surfactant, sodium azide (< 0.1%) and preservatives	Store the reagents upright at 2–8° C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability–60 days.
ADVIA Centaur HBsAg II calibrator vials	2.5 mL/vial high calibrator: purified human HBsAg in buffer with sodium azide (< 0.1%); low calibrator: buffer with sodium azide (< 0.1%)	Store at 2–8° C. Protect vials from all heat sources.	Until the expiration date on the vial. Onboard stability–8 hours.
ADVIA Centaur HBsAg quality control material vials[a]	10.0 mL/vial recalcified human plasma negative and positive for HBsAg with preservatives	Store at 2–8° C. Protect vials from all heat sources.	Until the expiration date on the vial. Onboard stability–8 hours.

Reagent	Description	Storage	Stability
ADVIA Centaur APW 1 ReadyPack ancillary reagent pack; Ancillary Probe Wash 1[a]	25.0 mL/pack 0.4N sodium hydroxide	Store the reagents upright at 2–8° C. Protect reagent packs from all heat sources.	Until the expiration date on the pack label. or Onboard stability–14 days.
ADVIA Centaur Wash 1 [redacted]	1500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25° C.	Until the expiration date on the vial. Onboard stability–1 month.
ADVIA Centaur Wash 1 [redacted]	2500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25° C.	Until the expiration date on the vial. Onboard stability–1 month.

a. See *Materials Required but Not Provided*.

Warnings and Precautions

For *in vitro* diagnostic use.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

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CAUTION! POTENTIAL BIOHAZARD

Contains human source material. Each donation of human blood or blood component was tested by FDA-approved methods for the presence of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), as well as for hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (HCV). The test results were negative (not repeatedly reactive). No test offers complete assurance that these or other infectious agents are absent; this material should be handled using good laboratory practices and universal precautions.[6-8]

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 and high calibrator contain human plasma that is reactive for HBsAg. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Note Some components of this product contain sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

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 prevailing regulatory requirements.

Preparing Reagents

Reagents are liquid and ready to use. 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, see the system operator's guide.

Storage and Stability

Note Discard reagent packs at the end of the 60-day onboard stability interval. Do not use reagents beyond the expiration date.

Protect reagent packs from all heat and light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2° to 8° C away from heat and light sources.

Store reagent packs upright.

Specimen Collection and Handling

Serum and plasma (EDTA, lithium-heparin, or sodium-heparin) are the approved sample types for this assay. Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HBsAgII 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.

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS),[9] and augmented with additional sample handling studies using the ADVIA Centaur HBsAgII 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 samples where the centrifugation step was varied up to 24 hours post-draw, no clinically significant differences were observed.
- Test samples as soon as possible after collecting.
- Store primary tube samples at 2° to 8° C up to 14 days. Keep samples stoppered 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 14 days, no clinically significant differences were observed.

- Store samples stoppered at all times at 2° to 8° C up to 14 days.
- 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 4 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 1 day or refrigerated up to 7 days demonstrated no qualitative differences. Store samples stoppered at 2° to 8° C upon arrival. If during shipment, samples may be subjected to temperatures above 25° C, then ship samples frozen.

Procedure

Materials Provided

REF	Contents	Number of Tests
10492138	1 ReadyPack primary reagent pack containing ADVIA Centaur HBsAgII Solid Phase and Lite Reagent	200
	1 Ancillary pack containing ADVIA Centaur HBsAgII Ancillary Pack Reagent ANC	
	ADVIA Centaur HBsAgII calibrators 2 x 2.5 mL low calibrator CAL L 2 x 2.5 mL high calibrator CAL H ADVIA Centaur HBsAgII Master Curve card ADVIA Centaur HBsAgII Calibrator Assigned Value cards	

Materials Required but Not Provided

Item (REF)	Description	
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 1 WASH 1	2 x 1500 mL/pack
03773025	ADVIA Centaur Wash 1 WASH 1 [a]	2 x 2500 mL/pack
03395373	ADVIA Centaur Ancillary Probe Wash 1 APW 1	2 Readypack ancillary reagent packs containing 25.0 mL/pack

a. For use with systems with 2500 mL capacity.

Assay Procedure

For detailed instructions on performing the procedure, refer to the system operating instructions or to the online help system.

The system automatically performs the following steps:

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

Preparing the System

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.

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.

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

Preparing the Samples

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 the system operating instructions.

Before placing samples on the system, ensure that samples have the following characteristics:

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

Performing Calibration

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

Note 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.

Additionally, the ADVIA Centaur HBsAgII 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.

Master Curve Calibration

The ADVIA Centaur HBsAgII 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 bar-code 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.

Using Bar-code Labels

Calibrator bar-code labels are lot-number specific. Do not use bar-code labels from one lot of calibrators with any other lot of calibrators.

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

Calibration Procedure

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values

using the bar-code scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

Perform the calibration procedure using the following steps:

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

1. Schedule the calibrators to the worklist.

2. Label 2 sample cups with calibrator bar-code labels: 1 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.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

For quality control of the ADVIA Centaur HBsAgII 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 Bar-code Labels

Note Control bar-code labels are lot-number specific. Do not use bar-code labels from 1 lot of controls with any other lot of controls.

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

Quality Control Procedure

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.

Perform the quality control procedure using the following steps:

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

1. Schedule the quality control samples to the worklist.
2. Label 2 sample cups with quality control bar-code labels: 1 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 or within the laboratory's established values, do not report results. Take the following actions:

- Consider the sample results invalid and repeat testing.
- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

Results

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

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 HBsAgII 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% CI 97.5 - 100%) of 145 available positive specimens with ADVIA Centaur HBsAgII Index values greater than 50.00 were confirmed positive for the presence of HBsAg .

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

- Samples with an Index Value of less than 1.0 are considered nonreactive (negative) for HBsAg.
- Samples with an Index Value of greater than or equal to 1.0 but less than or equal to 50 are considered reactive (positive) for HBsAg. The test must be repeated in duplicate. If 2 of the 3 results are nonreactive, the sample is considered negative for HBsAg. If at least 2 of the 3 results are reactive, the sample is repeatedly reactive, and the presence of HBsAg should be confirmed with the ADVIA Centaur HBsAg Confirmatory assay, additional HBV marker assays, or another approved confirmatory method.
- If the sample is greater than 50 or flagged as "> Index Range," the specimen is reactive for HBsAg, and no further testing is required.

  Note When the ADVIA Centaur HBsAgII 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), it is suggested that the ADVIA Centaur HBsAg Confirmatory assay be used to confirm the result.
- Sample results are invalid and must be repeated if the controls are out of range.

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.

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Limitations

The following information pertains to limitations of the assay:

- The ADVIA Centaur HBsAgII assay is limited to the detection of HBsAg in human serum and plasma (EDTA, lithium-heparin, or sodium-heparin).
- Assay performance characteristics have not been established when the ADVIA Centaur HBsAgII assay is used in conjunction with other manufacturers' assay for specific HBV serological markers.
- The performance of the ADVIA Centaur HBsAgII 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.

- For diagnostic purposes, the ADVIA Centaur HBsAgII test results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.
- 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. 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.
- 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.
- A reactive HBsAg result does not exclude co-infection by another hepatitis virus

Expected Values

The total prospective study population for the ADVIA Centaur HBsAgII testing consisted of 2119 patients. Of the 2119 patients, collected prospectively, 1044 patients

(49.3%) were from the high risk population, 818 patients (38.6%) were from the signs

and symptoms population, 83 patients (3.9%) were from the dialysis population, 20

(0.9%) patients were transplant recipients, 86 were pregnant female (4.1%) and 68 were

pediatric samples (3.2%). The study population was 19.9% Caucasian, 58.6% Black,

18.5% Hispanic, 1.8% Asian, 0.4 % American Indian/Alaskan native, and 0.8% other

. The majority of the patients were male (61.8% male,

38.2% female). The mean age was 46 years (range of 11 to 92). Patients in the

prospective study population were from the following geographic regions: Alabama

(0.1%), California (5.0%), Colorado (0.1%), District of Columbia (22.7%), Florida

(57.8%), Georgia (0.1%), Maryland (3.0%), Texas (10.7%) and Virginia (0.2%).

The ADVIA Centaur HBsAgII 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	Nonreactive [a]		Reactive [b]		Total	
		(N)	(%)	(N)	(%)	(N)	(%)
10–19	Female	17	100.0		0.0	17	53.1
	Male	15	100.0		0.0	15	46.9
	Total	32	100.0		0.0	32	100.0
20–29	Female	110	98.2	2	1.8	112	47.5
	Male	121	97.6	3	2.4	124	52.5
	Total	231	97.9	5	2.1	236	100.0
30–39	Female	124	93.9	8	6.1	132	45.1
	Male	148	91.9	13	8.1	161	54.9
	Total	272	92.8	21	7.2	293	100.0
40–49	Female	251	96.2	10	3.8	261	36.9
	Male	414	92.6	33	7.4	447	63.1
	Total	665	93.9	43	6.1	708	100.0
50–59	Female	201	92.6	16	7.4	217	32.5
	Male	414	92.0	36	8.0	450	67.5
	Total	615	92.2	52	7.8	667	100.0
60–69	Female	49	94.2	3	5.8	52	35.6
	Male	82	87.2	12	12.8	94	64.4
	Total	131	89.7	15	10.3	146	100.0

Age Range (Years)	Gender	Nonreactive [a] (N)	(%)	Reactive [b] (N)	(%)	Total (N)	(%)
≥ 70	Female	15	83.3	3	16.7	18	48.6
	Male	17	89.5	2	10.5	19	51.4
	Total	32	86.5	5	13.5	37	100.0
Total	**Female**	**767**	**94.8**	**42**	**5.2**	**809**	**38.2**
	Male	**1211**	**92.4**	**99**	**7.6**	**1310**	**61.8**
	Total	**1978**	**93.3**	**141**	**6.7**	**2119**	**100.0**

a Samples with an Index Value • 1.00 and initially reactive samples that did not confirm positive

b Samples with an Index Value ≥ • 1.00 were confirmed 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 (2119 patients total) was determined by serological

assessment using resultant hepatitis marker profiles obtained from results of

commercially available, FDA- approved ADVIA Centaur reference assays. The

serological assessment included the following 4 HBV markers: hepatitis B virus surface

antigen (HBsAg), total antibody to hepatitis B virus core antigen (anti-HBc Total), IgM

antibody to hepatitis B core antigen (anti-HBc IgM), and total antibody to hepatitis B virus

surface antigen (anti-HBs). Testing of these specimens occurred at each study site. The

individual ADVIA Centaur HBsAgII assay result was compared to the reference HBsAg 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(-) and equivocal patterns of the 4HBV 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 14 unique reference marker patterns observed using the ADVIA Centaur

HBsAgII assay. These patterns are presented in the following table:

Classification by Reference Markers (All Testing Sites)

	HBV Reference Markers			
HBV Classification	HBsAg	aHBcM	HBcT	aHBs
Early Acute	+	–	–	–
Acute	+	+	+	–
Late Acute	+	+	+	+
Chronic	+	–	+	+
Chronic	+	–	+	–
Chronic	+	–	–	+
Early Recovery	–	+	+	+
Early Recovery	–	Eqv	+	+
Early Recovery	–	+	+	–
Early Recovery	–	+	–	+
Immune due to Hepatitis vaccination	–	–	–	+
Immune natural infection	–	–	+	+
Recovered	–	–	+	–
Not previously infected	–	–	–	–

+ = Reactive

– = Nonreactive

Eqv = Equivocal

Pediatric/Adolescent Comparison

Pediatric samples were prospectively collected (N=68) and retrospectively collected

samples (N=94). The pediatric population analysis was stratified by three age groups:

5 to 22 months, 2 to 12 years, and 13 to 21 years.

Comparison of Results in the Pediatric Population (All Testing Sites)

	Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Age Range	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
5 to 22 months	7	0	0	0	7
2 to 12 years	58	0	0	0	58
13 to 21 years	95	0	0	2	97
Total	160	0	0	2	162

Percent Agreement in the Pediatric Population (All Testing Sites)

	Negative Agreement		Positive Agreement	
Age Range (years)	% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
5 to 22 months	100.0 (7/7)	59.0–100.00	– (0/0)	–
2 to 12 years	100.0 (58/58)	93.8–100.00	– (0/0)	–
13 to 21 years	100.0 (95/95)	96.2–100.00	100.0 (2/2)	15.8–100.00
Total	100.0 (160/160)	97.7–100.00	100.0 (2/2)	15.8–100.00

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference system
n = the number of nonreactive reference system results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference system
n = the number of reactive reference system results

Nine additional HBsAg-positive pediatric samples were retrospectively collected and tested. The population analysis was stratified by three age groups as in the previous tables.

Comparison of Results in the Positive Pediatric Population

	Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Age Range	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
5 to 22 months	0	0	0	0	0
2 to 12 years	0	0	0	1	1
13 to 21 years	3	0	0	5	8
Total	**3**	**0**	**0**	**6**	**9**

Percent Agreement in the Positive Pediatric Population

	Negative Agreement		Positive Agreement	
Age Range (years)	% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
5 to 22 months	– (0/0)	–	– (0/0)	–
2 to 12 years	– (0/0)	–	100.0 (1/1)	2.5–100.00
13 to 21 years	100.0 (3/3)	29.2–100.0	100.0 (5/5)	47.8–100.00
Total	**100.0 (3/3)**	29.2–100.0	**100.0 (6/6)**	**54.1–100.00**

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference HBsAg assay
n = the number of nonreactive reference HBsAg assay results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference HBsAg assay
n = the number of reactive reference HBsAg assay results

Neonatal Population

20 cord blood samples were retrospectively collected and tested in Tarrytown. All were nonreactive on both ADVIA Centaur and reference assay.

Comparison of Results in the Neonatal Population (Tarrytown Testing Site)

Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
20	0	0	0	20

Percent Agreement in the Neonatal Population (All Testing Sites)

Negative Agreement		Positive Agreement	
% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
100.0 (20/20)	83.2–100.0	– (0/0)	–

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference system
n = the number of nonreactive reference system results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference system
n = the number of reactive reference system result

A total of 5 additional neonatal or cord blood samples were retrospectively collected and spiked with HBsAg-positive human serum to target the low-positive end of the assay.

Comparison of Results in the Positive Neonatal Population

ADVIA Centaur HBsAgII Assay vs. Reference HBsAg Assay	
Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay	Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay

Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
0	2	0	3	5

Percent Agreement in the Positive Neonatal Population

Negative Agreement		Positive Agreement	
% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
0.0 (0/2)	0.2–84.2	100.0 (3/3)	29.2–100.0

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference HBsAg assay
n = the number of nonreactive reference HBsAg assay results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference HBsAg assay
n = the number of reactive reference HBsAg assay results

Comparison of Results: Prospective Population

1965 samples in the high risk, signs and symptoms, and dialysis populations were run using the ADVIA Centaur HBsAgII assay and a reference HBsAg assay for each HBV specimen classification. The following results were obtained:

Comparison of Results by HBV Classification (All Testing Sites)

	Reference HBsAg Nonreactive		Reference HBsAg Reactive		
	ADVIA Centaur HBsAgII Assay		ADVIA Centaur HBsAgII Assay		
HBV Classification	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
EarlyAcute	0	0	0	4	4
Acute	0	0	0	10	10
Late Acute	0	0	0	2	2
Chronic	0	0	0	116	116
Early Recovery	22	0	0	0	22
Recovered	214	2	3	3	222
Immune due to natural infection	531	1	1	1	534
Immune due to hepatitis B vaccination	331	0	0	1	332
Not previously infected	722	0	1	0	723
Total	**1820**	**3**	**5**	**137**	**1965**

Percent Agreement

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

Percent Agreement by HBV Classification (All Testing Sites)

	Negative Agreement		Positive Agreement	
HBV Classification	**% (x/n)[a]**	**95% CI**	**% (x/n)[b]**	**95% CI**
EarlyAcute	– (0/0)	–	100.0 (4/4)	39.8–100.0
Acute	– (0/0)	–	100.0 (10/10)	69.2–100.0
Late Acute	– (0/0)	–	100.0 (2/2)	15.8–100.0
Chronic	– (0/0)	–	100.0 (116/116)	96.9–100.0
Early Recovery	100.0 (22/22)	84.6–100.0	– (0/0)	–
Recovered	99.1 (214/216)	96.7–99.9	50.0 (3/6)	11.8–88.2
Immune due to natural infection	99.8 (531/532)	99.0–100.0	50.0 (1/2)	1.3–98.7
Immune due to hepatitis B vaccination	100.0 (331/331)	98.9–100.0	100.0 (1/1)	2.5–100.0
Not previously infected	100.0 (722/722)	99.5–100.0	0.0 (0/1)	0.0–97.5
Total	**99.8 (1820/1823)**	**99.5–100.0**	**96.5 (137/142)**	**92.0–98.8**

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference system
n = the number of nonreactive reference system results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference system
n = the number of reactive reference system results

Comparison of Results: Retrospective Population

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

Comparison of Results in the Acute and Chronic Population (All Testing Sites)

	Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Study Type	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
Acute	0	0	0	40	40
Chronic	0	0	0	116	116
Total	**0**	**0**	**0**	**156**	**156**

Percent Agreement

The agreement between the ADVIA Centaur HBsAgII assay and the reference HBsAg assay including the retrospective acute and chronic HBV infected population is summarized in the following table:

Percent Agreement in the Acute and Chronic Population (All Testing Sites)

	Negative Agreement		Positive Agreement	
Study Type	% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
Acute	– (0/0)	–	100.0 (40/40)	91.2–100.00
Chronic	– (0/0)	–	100.0 (116/116)	96.9–100.00
Total	– (0/0)	–	**100.0 (156/156)**	**97.7–100.00**

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference system
n = the number of nonreactive reference system results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference system
n = the number of reactive reference system results

Prenatal Population

Serum samples from pregnant women were prospectively collected (N=86) and retrospectively collected (N=234) from a U.S. prenatal population including 320 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 HBsAgII assay and the reference HBsAg assay, the performance of the ADVIA Centaur HBsAgII 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 HBsAgII 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 the Prenatal Population (All Testing Sites)

	Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Trimester	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
1st	91	0	1	1	93
2nd	109	0	0	0	109
3rd	118	0	0	0	118
Total	**318**	**0**	**1**	**1**	**320**

Percent Agreement in the Prenatal Population (All Testing Sites)

	Negative Agreement		Positive Agreement	
Trimester	% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
1st	100.0 (91/91)	96.0–100.0	50.0 (1/2)	1.3–98.7
2nd	100.0 (109/109)	96.7–100.0	–(0/0)	–
3rd	100.0 (118/118)	96.9–100.0	–(0/0)	–
Total	**100.0 (318/318)**	**98.8–100.0**	**50.0 (1/2)**	**1.3–98.7**

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the
a reference system
n = the number of nonreactive reference system results

b x = the number of ADVIA Centaur results that are reactive in agreement with the
b reference system
n = the number of reactive reference system results

Samples from an additional 23 pregnant women were retrospectively collected and spiked with HBsAg-positive human serum to target the low-positive end of the end of the assay. The analysis was stratified by trimester

Comparison of Results in the Additional Positive Prenatal Population

	Reference HBsAg Nonreactive ADVIA Centaur HBsAgII Assay		Reference HBsAg Reactive ADVIA Centaur HBsAgII Assay		
Trimester	Nonreactive N	Reactive N	Nonreactive N	Reactive N	Total N
1st	0	4	0	6	10
2nd	0	2	0	4	6
3rd	0	2	0	5	7
Total	**0**	**8**	**0**	**15**	**23**

Percent Agreement in the Additional Positive Prenatal Population

	Negative Agreement		Positive Agreement	
Trimester	% (x/n)[a]	95% CI	% (x/n)[b]	95% CI
1st	0.0 (0/4)	0.0–100.0	100.0 (6/6)	54.1–100.0
2nd	0.0 (0/2)	0.0–100.0	100.0 **(4/4)**	39.8–100.0
3rd	0.0 (0/2)	0.0–100.0	100.0 **(5/5)**	47.8–100.0
Total	**0.0 (0/8)**	0.0–**100.0**	100.0 **(15/15)**	78.2–100.0

a x = the number of ADVIA Centaur results that are nonreactive in agreement with the reference HBsAg assay
n = the number of nonreactive reference HBsAg assay results

b x = the number of ADVIA Centaur results that are reactive in agreement with the reference HBsAg assay
n = the number of reactive reference HBsAg assay results

Seroconversion Panels

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

	HBsAg Positive Result From Initial Draw Date		Reference Assay vs ADVIA Centaur Assay
Panel ID	Reference Assay (Days)	ADVIA Centaur HBsAgII Assay (Days)	Difference in Bleed Days [a] (Numbers) [b]
11002	9	7	+2 (+1)
11007	36	36	0
11008	79	69	+10 (+2)
11011	110	103	+7 (+2)
11012	18	18	0
11016	27	27	0
11017	42	40	+2 (+1)
6280	13	13	0
6283	29	29	0
PHM925	8	4	+4 (+1)

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

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

High-Dose Hook Effect

Patient samples with high levels of HBsAg can cause a paradoxical decrease in the RLUs (high-dose hook effect). In this assay, patient samples with levels of HBsAg as high as 2.79 mg/mL are reactive.

Analytical Sensitivity

To examine the analytical sensitivity of the ADVIA Centaur HBsAgII assay, the WHO International Reference Standard, 00/588 was used to prepare a dilution series that was assayed using 2 ADVIA Centaur HBsAgII reagent lots. Linear regression was used to determine the concentration of WHO reference sample that corresponds to the ADVIA Centaur HBsAgII cut-off point (Index Value=1.00). The WHO InternationalUnit (IU) concentration at the assay cut-off point is 0.040 IU/mL.

Precision

The Centaur HBsAg II assay is designed to have a Within Run Precision CV of ≤ 10% and total CV of ≤ 12% for the samples targeted to >/=2 Index and to have a Within Run CV of ≤ 12% and total CV of ≤ 15%for samples targeted to 0.5-2 Index.

Precision was evaluated according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) protocol EP5-A2.[15] Samples were assayed in 2 replicates twice a day for 20 days. The following testing was performed at Siemens Healthcare Diagnostics on 2 ADVIA Centaur instruments on 2 reagent lots. The results shown below were obtained on one ADVIA Centaur system:

			Within Run		Among Run		Among Date		Among Lot		Total	
Sample	**N**	**Mean**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**
Positive Control	162	4.04	0.12	3.0	0.07	1.7	0.11	2.8	0.07	1.8	0.19	4.8
Serum 1	160	0.64	0.06	9.0	0.04	5.6	0.03	4.4	0	0	0.07	11.5
Serum 2	160	1.34	0.05	4.1	0.05	3.7	0.04	3.0	0.02	1.4	0.09	6.4
Serum 3	160	19.08	0.33	1.8	0.30	1.6	0.46	2.4	0	0	0.64	3.3
Serum 4	160	209.52	2.82	1.3	3.86	1.8	4.17	2.0	7.75	3.7	10.01	4.8
Serum 5	160	700.61	10.83	1.5	13.05	1.9	10.73	1.5	20.73	3.0	28.86	4.1
K2 EDTA 1	160	0.70	0.05	7.7	0.04	5.7	0.04	5.9	0.02	3.5	0.08	11.7
K2 EDTA 2	160	1.21	0.06	4.8	0.07	6.1	0	0	0.05	3.9	0.11	8.7
K2 EDTA 3	160	18.29	0.32	1.8	0.76	4.2	0.34	1.9	0	0	0.90	4.9
K2 EDTA 4	160	202.82	3.53	1.7	8.48	4.2	3.56	1.8	6.45	3.2	11.77	5.8
K2 EDTA 5	160	682.39	10.83	1.6	26.90	3.9	14.54	2.1	16.00	2.3	36.17	5.3
Lithium heparin 1	160	0.77	0.06	7.6	0.03	3.9	0.04	5.7	0	0	0.08	10.3
Lithium heparin 2	160	1.25	0.06	4.8	0.04	3.1	0.04	3.5	0	0	0.08	6.7
Lithium heparin 3	160	19.64	0.37	1.9	0.46	2.3	0.29	1.5	0.26	1.3	0.71	3.6
Lithium heparin 4	160	205.34	3.17	1.5	4.05	2.0	3.26	1.6	9.53	4.6	11.31	5.5
Lithium heparin 5	160	677.41	10.84	1.6	11.10	1.6	10.97	1.6	24.70	3.6	31.16	4.6
Sodium heparin 1	160	0.80	0.05	6.3	0.05	5.8	0.03	3.9	0.01	1.9	0.08	9.6
Sodium heparin 2	160	1.30	0.07	5.5	0.06	5.0	0.01	0.9	0.02	1.4	0.10	7.6
Sodium heparin 3	160	17.76	0.32	1.8	0.35	2.0	0.31	1.8	0.09	0.5	0.57	3.2
Sodium heparin 4	160	198.61	2.91	1.5	4.33	2.2	3.28	1.7	8.29	4.2	10.33	5.2
Sodium heparin 5	160	660.51	11.05	1.7	12.81	1.9	12.62	1.9	20.95	3.2	29.74	4.5

System Reproducibility

System reproducibility was determined by testing an 8 member panel and controls using 3 reagent lots, on 3 instruments at 3 sites over 5 days. Panel members were run in replicates of 4 on each day. Samples with out-of-range results are not listed in the reproducibility table. The following results were obtained:

ADVIA Centaur HBsAgII Reproducibility Estimates Across All Three Sites and All Three Reagent Lots

	Within-Run	Between-Run	Between-Lot	Between-Site	Total

Sample	N	Mean HBsAgII Index	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Positive Control	240	4.26	0.18	4.3	0.07	1.7	0.24	5.5	0.03	0.6	0.32	7.4
Serum 2	360	0.70	0.09	13.0	0.03	4.6	0.00	0.0	0.04	5.5	0.12	17.3
Serum 3	360	1.19	0.10	8.2	0.03	2.6	0.01	0.6	0.03	2.6	0.12	10.4
Serum 4	360	2.73	0.12	4.6	0.06	2.1	0.07	2.7	0.01	0.5	0.17	6.2
Serum 5	360	23.73	0.58	2.4	0.41	1.7	0.05	0.2	0.03	0.1	0.93	3.9
Serum 6	360	47.10	0.96	2.0	0.85	1.8	0.40	0.9	0.12	0.3	1.75	3.7
Serum 7	360	243.07	4.89	2.0	4.39	1.8	5.92	2.4	1.30	0.5	11.01	4.5
Serum 8	360	799.20	15.00	1.9	13.75	1.7	12.18	1.5	13.07	1.6	33.69	4.2

Cross-Reactivity

The ADVIA Centaur HBsAgII assay was evaluated for potential cross-reactivity with specimens from various clinical and disease states. Samples for different disease states were obtained based on the seropositive status for the respective condition. All samples were tested using the ADVIA Centaur HBsAgII assay. Samples that were positive on the Centaur HBsAgII assay were confirmed with the Centaur HBsAg confirmatory assay. The following results were obtained using the ADVIA Centaur HBsAgII assay:

		ADVIA Centaur HBsAgII Results	
Clinical Category	Number Tested	Nonreactive	Reactive
Rubella	10	10	0
VZV	10	10	0
Systemic Lupus Erythematosus (SLE)	10	10	0
Anti-Nuclear Antibody (ANA)	10	10	0

EBV	10	10	0
Herpes Simplex Virus-1/2	11	11	0
HBcTotal	10	6	4[a]
HCV	10	10	0
HIV-1	10	10	0
HAV	10	10	0
CMV IgM	10	10	0
CMV IgG	10	10	0
Flu vaccine recipient	10	10	0
Toxoplasma	10	10	0
HAMA	82	82	0
Rheumatoid Factor	53	53	0
Syphilis	10	10	0
HTLV I/II	10	10	0
Non-Viral Liver Disease	10	10	0
Multiparity	25	25	0
Total Samples	**306**	**302**	**4**

a Confirmed positive with the ADVIA Centaur HBsAg Confirmatory Assay

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
cholesterol	400 mg/dL of cholesterol
proteinemic (high)	12 g/dL of total protein

proteinemic (low)	4 g/dL of total protein[a]
hyper IgG	6 g/L of immunoglobulin G
biotin-spiked	10 ng/mL of biotin

a Demonstrates ≤ 10% change in results with protein as low as 4 g/dL

Interference testing was determined according to CLSI Document EP7-A2.[16]

In addition, the following bacterial and viral antigens were spiked into HBsAg nonreactive and reactive serum specimens: *S. aureus*, *P. aeruginosa, C. albicans, 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.

ADVIA Centaur HBsAgII results of various bacterial spikes

Spiked Material	Amount (CFU/mL)	Reactivity before spike	Reactivity after spike
S. aureus	1000	Nonreactive	Nonreactive
S. aureus	10,000	Nonreactive	Nonreactive
E. coli	1000	Nonreactive	Nonreactive
E. coli	10,000	Nonreactive	Nonreactive
C. albicans	1000	Nonreactive	Nonreactive
C. albicans	10,000	Nonreactive	Nonreactive
P. aeruginosa	1000	Nonreactive	Nonreactive
P. aeruginosa	10,000	Nonreactive	Nonreactive
S. aureus	1000	Reactive	Reactive
S. aureus	10,000	Reactive	Reactive
E. coli	1000	Reactive	Reactive
E. coli	10,000	Reactive	Reactive
C. albicans	1000	Reactive	Reactive
C. albicans	10,000	Reactive	Reactive
P. aeruginosa	1000	Reactive	Reactive
P. aeruginosa	10,000	Reactive	Reactive

ADVIA Centaur HBsAgII results of various viral antigen spikes

Spiked Material	Amount	Reactivity before spike	Reactivity after spike
EBV	1 μg/mL	Nonreactive	Nonreactive
EBV	1 ng/mL	Nonreactive	Nonreactive
CMV	1 μg/mL	Nonreactive	Nonreactive
CMV	1 ng/mL	Nonreactive	Nonreactive
Rubella	1 μg/mL	Nonreactive	Nonreactive
Rubella	1 ng/mL	Nonreactive	Nonreactive
VZV	1 μg/mL	Nonreactive	Nonreactive
VZV	1 ng/mL	Nonreactive	Nonreactive
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

Mutant HBsAg Detection

Although HBV is a DNA virus, it contains a polymerase that lacks proofreading activity, so error frequencies are comparable to those seen for retroviruses and other RNA viruses. Owing to the low fidelity of the polymerase, the high replication rate and overlapping reading frames, mutations occur throughout HBV genome.[17] The mutations of diagnostic concern are those that occur in the sequence coding for “a” determinant (amino acids 124-147) within the major hydrophilic loop (amino acids 100-170) of the HBV surface antigen (HBsAg).[18] All immunoassays for HBsAg have antibodies that bind in this region and amino acid changes in this region can lead to false negative results when these changes occur at specific antibody binding sites.[19] Patients with Chronic HBV Infections are the primary source of mutants due to length of infection and increase in selective pressures that include immunization (active or passive) and drug

treatment and the majority of problematic HBsAg mutations are found in this population.

Using recombinant DNA techniques, a series of HBsAg mutants was produced. All sequences were evaluated by DNA sequence analysis to confirm the mutations. The recombinant mutants were expressed in insect cells using the baculovirus expression system. The cell lysates were analyzed by the ADVIA Centaur HBsAgII assay. One native mutant HBsAg plasma sample obtained from a positive patient specimen (S143L) was also included in the study.

The 24 recombinant mutant HBsAg samples shown below were tested and found reactive with the ADVIA Centaur HBsAgII assay. These mutant HBsAg samples represent the most common HBsAg mutants reported in the literature.[20–22]

Mutation Type	Amino Acid Position Substitution
Single	Y161H, C124R, C137W, D144A, F134H, F134N, G130D, G145R, K122I, K122T, M133L, Q129H, T123N, T126S, T131N, T143L, T143M
Double	K122N + G145R, T123N + G145R
Triple	M133I + F134H + D144V, T126S + Q129H + M133L
Insertion	122RA123, 122RG123, 123RGT124

Technical Assistance

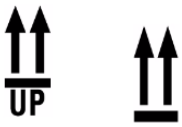
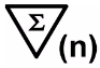
For customer support, contact your local technical support provider or distributor.

www.siemens.com/diagnostics

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community

	CE Mark		CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
	Do not freeze (> 0° C)		Temperature limitation (2–8° C)
	Lower limit of temperature (≥ 2° C)		Upper limit of temperature (≤ -10° C)
	Keep away from sunlight and heat		Up
	Use by		Contains sufficient for (n) tests
	Batch code		Shake the reagent pack vigorously. Refer to *Preparing Reagents* in the assay-specific ADVIA Centaur product instructions for detailed information.
YYYY-MM-DD	Date format (year-month-day)	Rev.	Revision
	Green dot		Printed with soy ink

Recycle

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