Bayer HealthCare

QC

CE 0088

HBc IgM (aHBcM)

Contents

REF	Contents
00504945	2 vials of Negative Control CONTROL ⊖
	2 vials of Positive Control CONTROL ⊕
	Expected Values Card and barcode labels

Preliminary xxxxxxxx Rev. A, 2004-05

Intended Use

For *in vitro* diagnostic use in monitoring the performance of the HBc IgM assay on the ADVIA Centaur® Systems. The performance of the HBc IgM quality control material has not been established with any other anti-HBc IgM assays.

Control Description

Volume	Ingredients	Storage	Stability
7.0 mL/vial	Processed human plasma negative and positive for IgM antibodies to HBc antigen with preservatives	2–8°C	Until the expiration date on the vial label or onboard–8 hours

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. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

For *In Vitro* Diagnostic Use.

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 HBc IgM quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HBc IgM 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 4 to 5 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.

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 HBc IgM quality control material. The expected values are traceable to the standardization of the HBc IgM assay. For additional information, refer to the reagent instructions for use.

The expected values should be used only as a guide in evaluating performance. Since performance is subject to the design and condition of each instrument or reagent system, it is recommended that each laboratory establish its own expected values and acceptable limits. 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* or within the laboratory's established 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

The results obtained using the HBc IgM 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.

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

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.

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.

HBc IgM (aHBcM)

DRAFT – 8/11/04

Assay for the Detection of IgM Antibodies to Hepatitis B Core Antigen

Assay Summary

Sample Type	*Serum, EDTA plasma, Lithium or sodium heparinized plasma*
Sample Volume	*15 µl*
Calibrator	*HBc IgM*

Contents

Catalog Number	Contents	Number of Tests
00504619	1 ReadyPack® primary reagent pack containing ADVIA Centaur® HBc IgM Lite Reagent and Solid Phase 1 Ancillary pack containing ADVIA Centaur HBc IgM Ancillary Reagent ADVIA Centaur HBc IgM Master Curve card 1 vial HBc IgM Low Calibrator 1 vial HBc IgM High Calibrator ADVIA Centaur HBc IgM Calibrator Assigned Value card	100

Intended Use

The ADVIA Centaur HBc IgM assay is an *in vitro* diagnostic test for the qualitative determination of IgM response to hepatitis B virus core antigen in human serum and plasma (EDTA or lithium or sodium heparinized) using the ADVIA Centaur® System. The assay uses recombinant HBc antigen. This assay may be used in combination with other hepatitis B virus (HBV) marker assays to define the clinical status of known HBV infected patients or can be combined with other HBV, HAV (hepatitis A virus), and HCV (hepatitis C virus) assays for the diagnosis of patients presenting symptoms of acute viral hepatitis.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants or children.

Assay performance characteristics have not been established when the ADVIA Centaur HBc IgM assay is used in conjunction with other manufacturers' assays for specific HBV serological markers.

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.

It is recommended that specimen results be reported only as nonreactive, reactive, or equivocal. The values reported by this assay are arbitrary and do not relate to an endpoint antibody level

Materials Required But Not Provided

Catalog Number	Description	Contents
00504945	ADVIA Centaur HBc IgM quality control material	2 x 7.0 mL Negative Control 2 x 7.0 mL Positive Control Expected Value card
01137199 (112351)	ADVIA Centaur Wash 1	2 x 1500 mL/pack

Summary and Explanation of the Test

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.[1,2]

The average incubation period for HBV infection is 6 to 8 weeks (range 1 to 6 months). Infection is usually asymptomatic. 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.[1,2,3]

Anti-HBc IgM titers increase rapidly, peak during the acute infection stage of HBV infection and then fall to a relatively low level as the patient recovers or becomes a chronic carrier. Anti-HBc IgM titers are useful in the diagnosis of acute HBV infection even when HBsAg concentrations are below the sensitivity of the diagnostic assay. Anti-HBc IgM may be the only specific marker for the diagnosis of acute HBV infection. The use of other viral markers such as HBsAg, anti-HBs, and anti-HBc total to differentiate acute from chronic hepatitis B is inconclusive because most of these markers are also seen in chronic infection.[1,4,5]

Assay Principle

The ADVIA Centaur HBc IgM assay is an IgM capture immunoassay using a 2-step format. The Ancillary Reagent contains biotinylated anti-human IgM. The Solid Phase contains streptavidin coated microparticles. In the Lite Reagent, recombinant HBc antigen (full length, grown in yeast) is combined with anti-HBc labeled with acridinium ester.

The sample is incubated with the Ancillary Reagent. The Solid Phase is added next and the streptavidin coated microparticles in the Solid Phase bind the IgM. After a wash step, the Lite Reagent is added. Antibody-antigen complexes will form if anti-HBc IgM is present in the sample.

The system automatically performs the following steps:

- dispenses 15 µL of sample into a cuvette
- dispenses 200 µL of Ancillary Reagent and incubates for 6 minutes at 37°C
- dispenses 250 µL of Solid Phase and incubates for 18 minutes at 37°C
- Washes the cuvette with Wash 1, followed by a 6.75 minute incubation at 37°C
- Dispenses 95 µL of Lite Reagent, 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. Assay results above the assay's cutoff are not indicative of antibody level. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Specimen Collection and Handling

Serum, EDTA plasma, lithium or sodium heparinized plasma are the recommended sample types for this assay.

Heparin has been shown to increase the Index values in some HBc IgM reactive samples by up to 28% relative to serum. Results obtained from heparin specimens falling near the cutoff should be repeated with a serum specimen or interpreted with caution.

Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HBc IgM 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, or pleural fluids.

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 HBc IgM 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 samples at 2 to 8°C if not tested within 8 hours of collection.
- Store primary tube samples at 2 to 8°C up to 2 days. 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.
- Store samples stoppered and upright at all times at 2 to 8°C up to 2 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 2 days or refrigerated up to 3 days demonstrated no qualitative differences. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 2 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: 1500 x g for 10 minutes; follow tube manufacturer's recommendations [6])
- 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*.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur HBc IgM ReadyPack primary reagent pack	Lite Reagent	9.5 mL/ reagent pack	recombinant hepatitis B core antigen (~0.370 µg/mL) combined with acridinium ester labeled monoclonal mouse anti-HBc (~0.037 µg/mL) in buffer with bovine serum albumin, 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.*
	Solid Phase	25.0 mL/ reagent pack	streptavidin coated paramagnetic microparticles in buffer with bovine serum albumin, 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.*
ADVIA Centaur HBc IgM Ancillary Reagent Readypack ancillary reagent pack	Ancillary Reagent	20.0 mL/ reagent pack	biotinylated monoclonal mouse anti-human IgM (~0.375 µg/ mL) in buffer with bovine serum albumin, 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.*
HBc IgM calibrator vials	Calibrators	2.0 mL/ vial	processed human plasma positive for IgM antibodies to HBc antigen with preservatives	2–8°C	until the expiration date on the vial or onboard 8 hours
HBc IgM quality control material vials*	Controls	7.0 mL/ vial	processed human plasma negative and positive for IgM antibodies to HBc antigen 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*

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 (BSL 2).[7-9]

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 calibrators 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.

Loading Reagents

Ensure that the system has sufficient primary 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 Ancillary Reagent provided in this kit is matched to the Solid Phase and Lite Reagent. Do not mix Ancillary Reagent lots with different lots of Solid Phase and Lite Reagent.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	28 days

Additionally, the ADVIA Centaur HBc IgM 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 HBc IgM assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Ancillary Reagent. For each new lot number of Lite Reagent, Solid Phase, and Ancillary 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 HBc IgM assay, use ADVIA Centaur HBc IgM 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 HBc IgM Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HBc IgM 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 4 to 5 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 HBc IgM assay, use ADVIA Centaur HBc IgM quality control materials. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and /or federal regulations or accrediting organizations.

NOTES: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, it is recommended to run a negative control and positive control close to the clinically relevant point (1.0 Index). The quality control furnished is in a defibrinated plasma, e.g., serum matrix. The user should provide alternate control material for plasma when necessary.

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 HBc IgM quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HBc IgM 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, it is recommended that quality control samples be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat and run 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 4 to 5 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 suggested Expected Values or within the laboratory's established values, then do the following:

- consider the sample results invalid and do not report result. Repeat sample testing with new controls.
- 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
- investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
- if necessary contact Bayer HealthCare for more assistance

Sample Volume

This assay requires 15 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 cutoff for the ADVIA Centaur HBc IgM assay was verified based on results of Receiver-Operator characteristics (ROC) Curve[10] and clinical agreement generated from clinical studies. The ROC analysis was performed using 791 specimens, 434 HBV negative donors & 357 HBV positive patients. Approximately 74% of the samples were in serum, the remaining 26% were in EDTA and heparin plasma.

The system reports HBc IgM results in Index Values and as reactive, equivocal, or nonreactive. Index values above the assay's cutoff are not indicative of the antibody level present in the sample.

- Samples with a calculated value of less than 0.80 Index Value are considered nonreactive for IgM antibodies to hepatitis B core antigen.
- Samples with a calculated value greater than or equal to 0.80 Index Value and less than 1.00 Index Value are considered equivocal and must be repeated. It is recommended that the test be repeated in duplicate and the results be reported based on the repeat results. If the results are still equivocal after repeat testing, obtain a new specimen and retest using the ADVIA Centaur HBc IgM assay.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for IgM antibodies to hepatitis B core antigen.
- Sample results are invalid and must be repeated if the controls are out of range.

CAUTION: Heparin has been shown to increase the Index values in some HBc IgM reactive samples by up to 28% relative to serum. Results falling near the cutoff should be repeated with a serum specimen or interpreted with caution.

Limitations

- The ADVIA Centaur HBc IgM assay is limited to the detection of IgM antibodies to hepatitis B core antigen in human serum or plasma (EDTA plasma, lithium or sodium heparinized plasma).
- Assay performance characteristics have not been established when the ADVIA Centaur HBc IgM assay is used in conjunction with other manufacturers' assays for specific HBV serological markers.
- The performance of the ADVIA Centaur HBc IgM 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, or pleural fluids.
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[11] 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 anti-HBc IgM result does not exclude co-infection by another hepatitis virus.

Expected Results

2021 patient samples were run in the ADVIA Centaur HBc IgM assay. The following results classified by gender and age range were obtained:

Age Range (Years)	Gender	Reactive (N)	Reactive (%)	Nonreactive (N)	Nonreactive (%)	Equivocal (N)	Equivocal (%)	Total
0-9	M	0	--	0		0	--	0
	F	0	--	0		0	--	0
10-19	M	0	--	7	100.00	0	--	7
	F	0	--	14	100.00	0	--	14
20-29	M	1	1.19	83	98.81	0	--	84
	F	2	2.00	98	98.00	0	--	100
30-39	M	2	1.02	193	98.47	1	0.51	196
	F	2	1.08	183	98.92	0	--	185
40-49	M	7	1.82	376	97.92	1	0.26	384
	F	1	0.34	295	99.33	1	0.34	297
50-59	M	6	2.08	281	97.57	1	0.35	288
	F	2	0.97	204	99.03	0	--	206
60-69	M	1	1.15	86	98.85	0	--	87
	F	1	1.01	98	98.99	0	--	99
>/=70	M	2	4.44	43	95.56	0	--	45
	F	0	--	28	100.00	0	--	28
Unknown	M	0	--	1	100.00	0	--	1
	F	0	--	0	--	0	--	0
Total		27	1.34	1990	98.46	4	0.20	2021

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[12]

Performance Characteristics

Results by Specimen Classification

2021 patients participated in a multi-site study to evaluate the clinical performance of the ADVIA Centaur HBc IgM assay. The group consisted of 54.03% males and 45.97% females and participants ranged in age from 12 to 82 years. The HBV disease classification for each patient was determined using a hepatitis marker profile consisting of serological reference assays for the detection of HBsAg, HBeAg, anti-HBc IgM, anti-HBc total, anti-HBe, and anti-HBs. Testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (34%), Dallas, TX (34%) and New York City, NY (33%). The individual 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.The specimen classification based on positive and negative patterns for the six HBV reference assays is listed in the following table:

HBV Classification	HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc Total	Anti-HBe	Anti-HBs (>10mIU/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	-	+	-	+	+	+

Comparison of Results

2021 samples were run in the ADVIA Centaur HBc IgM assay and an anti-HBc IgM reference assay for each HBV specimen classification The following results were obtained:

	Reference Anti- HBc IgM Assay Negative			Reference Anti- HBc IgM Assay Equivocal/Grey Zone			Reference Anti- HBc IgM Assay Positive			
HBV	ADVIA Centaur HBc IgM Assay			ADVIA Centaur HBc IgM Assay			ADVIA Centaur HBc IgM Assay			
Classification	Reactive	Nonreactive	Equivocal[a]	Reactive	Nonreactive	Equivocal[a]	Reactive	Nonreactive	Equivocal[a]	Total
Acute	0	0	0	0	0	0	2	0	1	3
Chronic	4	104	0	7	3	1	0	1	0	120
Early Recovery	3	108	0	1	0	0	2	1	0	115
Recovery	1	210	0	2	0	0	0	0	0	213
Recovered	1	326	1	2	0	0	0	0	0	330
HBV Vaccine Response	0	384	0	0	0	0	0	0	0	384
Not previously Infected	2	833	1	0	0	0	0	0	0	836
Uninterpretable	0	20	0	0	0	0	0	0	0	20
Total	11	1985	2	12	3	1	4	2	1	2021

a Equivocal results following repeat testing

Percent Agreement

The positive and negative percent agreement between the ADVIA Centaur HBc IgM assay and an anti-HBc IgM reference assay were calculated for each HBV specimen classification. Specimens which were equivocal in either the ADVIA Centaur HBc IgM assay (n=3) or the Reference anti-HBc IgM assay (n=15) were considered to be discrepant samples for the purpose of specimen characterization. Reference assay equivocal results were assigned the opposite clinical interpretation than that of the ADVIA Centaur result and ADVIA Centaur equivocal results were assigned the opposite clinical interpretation than that of the reference assay result. Four ADVIA Centaur nonreactive / reference assay reactive discrepants and fourteen ADVIA Centaur reactive / reference assay nonreactive discrepants resulted from the assignment of opposite interpretations to equivocal samples. The one sample which was equivocal in both assays is considered a negative concordant sample for the purpose of calculating % agreement. The following results were obtained:

HBV Classification	% Positive Agreement	95% Exact Confidence Interval (CI)	% Negative Agreement	95% Exact Confidence Interval (CI)
Overall	40.0 (4/10)	12.2 to 73.8	98.8 (1986/2011)	98.2 to 99.2
Acute	66.7 (2/3)	9.4 to 99.2	--	--
Chronic	0.0 (0/4)	0.0 to 60.2	90.5 (105/116)	83.7 to 95.2
Early Recovery	66.7 (2/3)	9.4 to 99.2	96.4 (108/112)	91.1 to 99.0
Recovery	--	--	98.6 (210/213)	95.9 to 99.7
Recovered	--	--	98.8 (326/330)	96.9 to 99.7
HBV Vaccine Response	--	--	100.0 (384/384)	99.0 to 100.0
Not Previously Infected	--	--	99.6 (833/836)	98.9 to 99.9
Uninterpretable	--	--	100.0 (20/20)	83.2 to 100.0

Comparison of Results, Retrospective Population

A population of commercially sourced HBV acute and HBV chronic samples was also tested using both the ADVIA Centaur HBc IgM assay and an anti-HBc IgM reference assay. Positive percent agreement for this population is 97.9% (46/47) and negative percent agreement for this population is 98.1% (105/107) when reference anti-HBc IgM assay equivocal samples are assigned interpretations the opposite of ADVIA Centaur interpretations. The following results were obtained.

	Reference Anti-HBc IgM Assay Negative			*Reference Anti-HBc IgM Assay Equivocal / Grey Zone*			*Reference Anti-HBc IgM assay Positive*			
HBV	*ADVIA Centaur® HBc IgM Assay*			*ADVIA Centaur® HBc IgM Assay*			*ADVIA Centaur® HBc IgM Assay*			
Classification	*Reactive*	*Nonreactive*	*Equivocal[a]*	*Reactive*	*Nonreactive*	*Equivocal[a]*	*Reactive*	*Nonreactive*	*Equivocal[a]*	*Total*
Acute	0	0	0	2	1	0	46	0	0	49
Chronic	0	105	0	0	0	0	0	0	0	105
Total	0	105	0	2	1	0	46	0	0	154

a Equivocal results following repeat testing

Seroconversion Panels

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

	Anti-HBc IgM Positive Result From Initial Draw Date		*Reference Assay vs. ADVIA Centaur Assay*
Panel ID	*Reference Assay (Days)*	*ADVIA Centaur Assay (Days)*	*Difference (Bleeds)*
RP-009	43	43	0
RP-016	60	60	0
RP-017	78	76	+1[a]
62433	43	41	+1
SB0413	78	78	0
22663D	69	69	0

a "+1" indicates that the ADVIA Centaur HBc IgM assay 1st reactive result was one bleed ahead of the first positive result for the reference HBc IgM assay.

Cross-Reactivity

The ADVIA Centaur HBc IgM assay was evaluated for potential cross-reactivity with viral antibodies and disease state specimens. The negative anti-HBc IgM status of each specimen was verified using an anti-HBc IgM reference assay. The following results were obtained using the ADVIA Centaur HBc IgM assay:

		ADVIA Centaur HBc IgM Results		
Clinical Category	Number Tested	Nonreactive	Equivocal	Reactive
Hepatitis A Infection (HAV)	9	9	0	0
Hepatitis C Infection (HCV)	12	12	0	0
Epstein-Barr Virus (EBV) IgM	10	10	0	0
Herpes Simplex Virus (HSV) IgG	10	10	0	0
Herpes Simplex Virus (HSV) IgM	10	10	0	0
Parvovirus IgM	5	5	0	0
Syphilis IgG	10	10	0	0
Syphilis IgM	10	10	0	0
Human Immunodeficiency Virus (HIV1/2)	10	10	0	0
VZV IgG	9	9	0	0
VZV IgM	6	6	0	0
Rubeola IgG	11	9	1[1]	1[2]
Non-viral Liver Disease	12	12	0	0
Rheumatoid Arthritis	12	11	0	1[3]
Anti-Nuclear Antibody (ANA)	6	6	0	0
Systemic Lupus Erythematosus (SLE)	3	3	0	0
Flu Vaccine Recipient	10	10	0	0
HAMA	9	9	0	0
Total Samples Tested	164	161	1	2

NOTE: Cross reactivity of samples with anti-yeast antibodies was not evaluated.

1. Sample had a 0.95 Index Value using the ADVIA Centaur HBc IgM assay and a 0.63 Index Value (Elevated Negative) using the reference HBc IgM assay.

2. Sample had a 1.39 Index Value using the ADVIA Centaur HBc IgM assay and a 0.86 Index Value (Equivocal) using the reference HBc IgM assay.

3. Sample had a 1.24 Index Value using the ADVIA Centaur HBc IgM assay and a 0.25 Index Value (Negative) using the reference HBc IgM 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	60 mg/dL of conjugated bilirubin
icteric	40 mg/dL of unconjugated bilirubin
proteinemic	12 g/dL of protein

Interference testing was determined according to NCCLS Document EP7-P.[13]

Precision

Precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-A.[14] Samples were assayed in three replicates twice a day for 20 days. The following results were obtained:

Sample	Mean Index Value	Within-run SD	Within-run %CV	Between -run SD	Between -run %CV	Total SD	Total %CV
Serum 1	0.09	0.00	0.8	0.00	0.6	0.00	1.0
Serum 2	0.66	0.02	3.6	0.00	0.0	0.03	3.9
Serum 3	1.26	0.07	5.4	0.00	0.0	0.08	6.3
Serum 4	2.15	0.09	4.3	0.05	2.1	0.11	5.2
Serum 5	5.06	0.33	6.6	0.17	3.4	0.38	7.6

System Reproducibility

The ADVIA Centaur HBc IgM reproducibility study was performed at 3 external sites using 2 reagent lots per site. A five member panel and controls were assayed in replicates of 5 on a single run per day over 6 days for each lot. The study was completed within a single calibration of the assay (one calibration interval).

The data from all 3 sites and from all 3 reagent lots were combined to obtain SD and percent CV for within run, between run, between testing site, between reagent lot, and total. The precision estimates were derived from variance component analysis. A NESTED SAS model was used for analysis. The reproducibility results are presented in the following table:

sample	Mean Index Value	Within Run SD	Within Run %CV	Between Run SD	Between Run %CV	Between Site SD	Between Site %CV	Between Lot SD	Between Lot %CV	Total SD	Total %CV
1	0.11	0.00	NA	0.00	NA	0.04	NA	0.01	NA	0.04	NA
2	0.82	0.04	5.4	0.02	2.5	0.03	3.5	0.01	1.4	0.06	7.0
3	1.68	0.10	6.0	0.07	4.3	0.00	0.0	0.01	0.8	0.12	7.4
4	1.41	0.10	7.0	0.07	4.7	0.00	0.0	0.13	9.4	0.18	12.6
5	5.93	0.59	9.9	0.20	3.3	0.47	8.0	0.00	0.0	0.78	13.1
Neg Control	0.10	0.00	NA	0.01	NA	0.05	NA	0.00	NA	0.06	NA
Pos Control	2.86	0.20	6.9	0.10	3.4	0.19	6.6	0.00	0.0	0.29	10.2

Technical Assistance

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

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