BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

ACCESS anti-HBc IgM
Hepatitis B Virus core IgM Antibody

REF D22920

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use only

Rx Only

FOR USE ON DXI 9000 ACCESS IMMUNOASSAY ANALYZERS

PRINCIPLE

CAUTION

U.S. federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical Laboratory; and use is restricted to, by, or on the order of a physician.

INTENDED USE

The Access anti-HBc IgM assay is a paramagnetic particle, chemiluminescent immunoassay for the in vitro qualitative detection of IgM antibodies to hepatitis B virus core antigen (anti-HBc IgM) in human pediatric (3 through 21 years) and adult serum and serum separator tubes or plasma [lithium heparin, lithium heparin separator tubes, dipotassium (K_2) EDTA, tripotassium (K_3) EDTA, sodium citrate, acid citrate dextrose (ACD), and citrate phosphate dextrose (CPD)] using the DxI 9000 Access Immunoassay Analyzer.

The Access anti-HBc IgM assay results may be used as an aid in the laboratory diagnosis of acute or recent hepatitis B virus (HBV) infection in individuals with signs and symptoms of hepatitis, when used in conjunction with other serological and clinical information.

The Access anti-HBc IgM assay is for use on the DxI 9000 Access Immunoassay Analyzer only.

This assay is not intended for the screening of blood, plasma, and cell or tissue donors.

SUMMARY AND EXPLANATION

Hepatitis B is a potentially life-threatening, chronic liver infection that is caused by the hepatitis B virus (HBV). Hepatitis B is a global health issue that can lead to cirrhosis and liver cancer.[1] HBV is a double-stranded DNA virus composed of two shells. Hepatitis B surface antigen (HBsAg) is embedded in the outer shell, and surrounds the hepatitis B core antigen (HBcAg)[2] which contains the viral genome, a relaxed-circular DNA and a polymerase that is responsible for the synthesis of viral DNA in infected cells. HBsAg plays a role in attaching to cell membranes, which initiates the infection process through an immune response that results in cell injury.[2,3] Hepatitis B e antigen (HBeAg), a soluble precore protein, is another HBV component.[4]

HBV is commonly spread through perinatal transmission from mothers to infants. Hepatitis B is also transmitted by needlestick injury, tattooing, piercing and exposure to infected blood and body fluids. Sexual transmission of hepatitis B can occur, particularly between unvaccinated men.[1] Identifying individuals with a Hepatitis B infection helps prevent transmission. The World Health Organization (WHO) recommends testing all donors (blood, plasma, organs) for hepatitis B to avoid transmission.[1]

With primary Hepatitis B infections, HBsAg can be detected in the blood 4 to 10 weeks after infection. This is followed by antibodies against the HBV core antigen (anti-HBc), which are mainly IgM antibodies in the early stages of infection.[3] A primary infection is either symptomatic or, more commonly, asymptomatic, particularly among children. For adults, most primary infections are self-limited. Less than 5% of infections do not resolve and develop into persistent infections, which can be either symptomatic or asymptomatic.[4,5] If the infection is symptomatic, the clinical manifestations of acute disease are typically expressed 2-3 months after viral exposure and can last for 2-4 months. Symptoms can include fatigue, poor appetite, nausea, vomiting, abdominal pain, low-grade fever, jaundice, dark urine and light stool color.[6]

Anti-HBc total (IgM and IgG) antibodies appear at the onset of symptoms in acute infection and persist for life. Therefore, they are markers for both acute and past infections.[6,7,8] Anti-HBc total, along with HBsAg and antibodies against HBsAg (anti-HBs), can be used to screen for hepatitis B infection.[9,10] Anti-HBc antibodies may be the only positive marker of an acute hepatitis B infection after HBsAg becomes negative and before the appearance of anti-HBs.[7] Anti-HBc total antibodies, when detected along with anti-HBs, indicate a resolved Hepatitis B infection. The appearance of anti-HBc total, with or without anti-HBs, can also indicate an individual whose Hepatitis B infection may reoccur within the context of immunosuppression.[8] Identifying anti-HBc IgM can further characterize an HBV infection, because anti-HBc IgM levels are high during an active infection and remain detectable for up to 6 months. Although the presence of anti-HBc IgM mainly differentiates between an acute and chronic HBV infection, anti-HBc IgM may also be present during flares in chronic hepatitis B.[8]

METHODOLOGY

Assay type: two-step enzyme immunoassay

The Access anti-HBc IgM assay is a two-step enzyme immunoassay. Paramagnetic particles coated with anti-human IgM monoclonal antibody and prediluted sample are added to a reaction vessel. After incubation, material bound to the solid phase is held in a magnetic field while unbound materials are washed away. HBc antigen complexed to anti-HBc monoclonal antibody alkaline phosphatase conjugate is added and the conjugate binds to the IgM antibodies captured on the particles. A second separation and wash step removes unbound conjugate.

A chemiluminescent substrate is then added to the vessel and light generated by the reaction is measured with a luminometer. The light production is compared to the cutoff value defined during calibration of the instrument. The qualitative assessment is automatically determined from a stored calibration.

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Stability				
Specimen	**Type**	**20 to 25°C (hours)**	**2 to 8°C (days)**	**-20°C or colder (days)**
Serum	Serum and Serum separator tube	72	7	30 Do not thaw samples more than 5 times.
Plasma	Lithium Heparin Lithium Heparin separator tube Dipotassium (K_2) EDTA Tripotassium (K_3) EDTA Sodium Citrate Acid Citrate Dextrose (ACD) Citrate Phosphate Dextrose (CPD)			

SPECIMEN COLLECTION AND PREPARATION

Blood Specimen

Blood Specimen

1. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[11,12] To minimize the effect of preanalytical factors observe the following recommendations for handling and processing blood samples:[11]
   A. Collect all blood samples observing routine precautions for venipuncture.
      - Follow blood collection tube manufacturer's recommendations for centrifugation.
      - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   B. Allow serum samples to clot completely before centrifugation in a vertical position, with the collection tube closure directed upwards.
      - Store non-anticoagulated collection tubes that contain gel separator in an upright position as soon as the mixing is complete.
      - Follow the tube manufacturer's recommendations for the length of serum/cells contact time before centrifuging samples. The clotting may be slower at cooler temperatures, or if the patient is on anticoagulant therapy.
   C. Frozen samples should be thawed at room temperature, mixed thoroughly, and centrifuged per tube manufacturer's recommendations prior to analysis.
2. Each laboratory should determine the acceptability of its own blood collection tubes and separation products that are in use. There may be variations in these products between manufacturers and between manufacturing lots.
3. Alternate collection types may be appropriate if the laboratory has established its own performance characteristics, as defined by applicable law.
4. Avoid assaying lipemic or hemolyzed samples.

REAGENTS

CONTENTS

Access anti-HBc IgM Reagent Pack

Ref. No. D22920: 200 determinations, 2 packs, 100 tests/pack

Well	Contents	Ingredients
R1a:	3.30 mL	Paramagnetic particles coated with streptavidin coupled to biotinylated anti-human IgM monoclonal antibody (mouse) in TRIS buffer, surfactant, protein (bovine), < 0.1% sodium azide and 0.1% ProClin* 300.
R1b:	5.80 mL	Phosphate buffer, HBc antigen (bacterial), surfactant, protein (bovine), < 0.1% sodium azide and 0.1% ProClin 300.
R1c:	5.80 mL	MES buffer, monoclonal anti-HBc antibody (mouse) alkaline phosphatase conjugate, surfactant, protein (bovine), < 0.1% sodium azide and 0.1% ProClin 300.
R1d:	2.00 mL	0.1M sodium hydroxide solution (NaOH)

*ProClin is a trademark of LANXESS Corp.

WARNING AND PRECAUTIONS

- **For *in vitro* diagnostic use.**
- Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices[13], regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local, state, and federal regulations and guidelines.
- This product contains material(s) of animal origin. Observe general safety guidelines for protection when handling this product.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

anti-HBc IgM Particles (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone < 0.05%
anti-HBc IgM Ancillary diluent (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.

	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone < 0.05%
anti-HBc IgM conjugate (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone < 0.05%
Sodium hydroxide 0.1M (Compartment R1d)	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Sodium hydroxide <0.5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access anti-HBc IgM Calibrator
   Provided as one positive level for anti-HBc IgM
   Ref. No. D22921
2. Access anti-HBc IgM QC
   Provided as three vials each of anti-HBc IgM positive control and anti-HBc IgM negative control
   Ref. No. D22922
3. Substrate:
   - Lumi-Phos PRO Substrate, Ref. No. B96000
4. Wash Buffer:
   - UniCel DxI Wash Buffer II, Ref. No. A16793

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	56 days

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., a broken elastomer), discard the pack.
- Discard reagent if any discoloration is observed.
- Do not freeze.

CALIBRATION

CALIBRATION INFORMATION

An active calibration is required for all tests. For the Access anti-HBc IgM assay, calibration is required every 56 days. See calibrator Instructions for Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Refer to Quality Control Instructions for Use.

Quality control materials are essential for monitoring the system performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[14] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

Include Access anti-HBc IgM QC materials that cover two levels of analyte, one negative and one positive. More frequent use of quality controls or the use of additional controls is left to the discretion of the operator, based upon good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstituting and storing controls. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results that were generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURE

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.
3. Mix the contents of a new (unpunctured) reagent pack by gently inverting the pack several times before loading it on the instrument. Do not invert an open (punctured) pack.
4. Use 10 µL of sample for each determination, in addition to the sample container and system dead volumes, when requesting the Access anti-HBc IgM assay.
5. The system default unit of measure for sample results is the Signal/Cutoff (S/CO) ratio.

LIMITATIONS

1. The Access anti-HBc IgM assay is intended for *in vitro* diagnostic use only.
2. This product is for use on the DxI 9000 Access Immunoassay Analyzer only.
3. Do not dilute samples as this could lead to incorrect results.
4. Do not use kits or components beyond the stated expiration date.
5. The Access anti-HBc IgM assay is limited to the detection of IgM antibodies to the hepatitis B virus core antigen in human serum, including serum separator tubes or plasma (lithium heparin, lithium heparin with gel, dipotassium (K_2) EDTA, tripotassium (K_3) EDTA, sodium citrate, acid citrate dextrose (ACD), and citrate phosphate dextrose (CPD)).
6. Do not use heat-treated samples or bodily fluids other than human serum or plasma such as saliva, urine, amniotic fluid or pleural fluid. The performance of this assay has not been evaluated using these specimen types and may result in inaccurate test results.
7. The performance of this test has not been established in cord blood, neonates or infants younger than 36 months of age.
8. When appropriate, performance characteristics of the assay may be affected in immunocompromised or immunosuppressed patients or other special populations.
9. For assays that employ antibodies, the possibility exists for interference by heterophile antibodies in the test sample. Patients who are regularly exposed to animals, or are subjected to medical treatments that utilize immunoglobulins or immunoglobulin fragments, may produce human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[15,16] These interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
10. Other potential interferences in the sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, fibrin, endogenous alkaline

phosphatase, exogenous alkaline phosphatase (e.g. asfotase alfa, Strensiq), and proteins capable of binding to alkaline phosphatase. Carefully evaluate results if the sample is suspected of having these types of interferences.[17,18]

11. A nonreactive anti-HBc IgM test result does not exclude the possibility of exposure to, or infection with hepatitis B virus. A nonreactive assay result may occur early during acute infection, prior to development of a host antibody response to infection, or when analyte levels are below the limit of detection of the assay. The Access anti-HBc IgM assay results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
12. Detection of the IgM type HBV antibodies to a single viral antigen indicates a present infection with hepatitis B virus but does not differentiate between acute infection and flares in chronic infection. Anti-HBc IgM may be detected during flares in chronic hepatitis B.[8] A reactive anti-HBc IgM result does not exclude co-infection by other types of hepatitis viruses.
13. Do not use specimens with obvious microbial contamination.
14. The calculated Signal/Cut-off (S/CO) values for anti-HBc IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. Values obtained with different assay methods should not be used interchangeably.
15. Avoid assaying grossly hemolyzed or lipemic samples.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. Results (Signal/Cutoff (S/CO)) are reported to be “reactive” or “nonreactive” as a function of their relationship with the “cutoff” (signal “greater than or equal to the cutoff” or “less than” the cutoff value). Test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

Result (S/CO)	Interpretation	Reporting Instructions
< 1.00	Nonreactive	Report result as nonreactive for anti-HBc IgM antibodies
≥ 1.00	Reactive	Report result as reactive for anti-HBc IgM antibodies

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

CLINICAL PERFORMANCE EVALUATION

Study Overview

A multi-center study was conducted using the DxI 9000 Access Immunoassay Analyzer to evaluate the performance of the Access anti-HBc IgM assay to detect anti-HBc IgM in plasma and serum samples from the intended use population. The overall study population included 2,537 specimens, consisting of 2,281 that were prospectively collected, and an additional 256 retrospective samples. Of the 2,281 specimens prospectively collected, 1,955 were from non-pregnant adults classified as increased risk for hepatitis due to lifestyle, behavior, occupation, or known exposure events, or individuals with signs and symptoms of hepatitis. 171 prospective specimens were from the pregnant population with increased risk and/or showed signs and symptoms of hepatitis. Specimens from the pregnant population included 71

from the first trimester, 67 from the second trimester, and 33 from the third trimester. In addition, 163 prospective and retrospective specimens were collected from the pediatric (ages 9-21) increased risk and/or sign & symptoms population. The table below summarizes the number of specimens in each population.

Cohort	Adult (Non-Pregnant) (n = 2,203)			Pregnant (n = 171)		Pediatric (Non-Pregnant) (n = 163)		
	S&S	IR	Retrospective	S&S	IR	S&S	IR	Retrospective
Prospective (n = 2,281)	192	1,763	0	12	159	2	153	0
Retrospective (n = 256)	0	0	248	0	0	0	0	8
Total (n = 2,537)	**192**	**1,763**	**248**	**12**	**159**	**2**	**153**	**8**

S&S = Signs and Symptoms, IR = Increased Risk

Expected Results

The prospective study population was 61.25% White, 26.30% Black or African American, 1.49% Asian, 1.89% American Indian or Alaska Native, 0.13% Native Hawaiian or other Pacific Islander, and 8.94% from unknown/other or unwilling to answer. 34.41% of the prospective study population was of Hispanic ethnicity. The majority of patients were female (59% female and 41% male). Patients in the prospective population were collected from the United States (US) in the following states: Arizona (585, 25.65%), California (26, 1.14%), Connecticut (123, 5.39%), Florida (284, 12.45%), Georgia (61, 2.67%), Idaho (71, 3.11%), Minnesota (68, 2.98%), North Carolina (1, 0.04%), New Jersey (91, 3.99%), New York (445, 19.51%), Ohio (8, 0.35%), Pennsylvania (59, 2.59%), South Carolina (33, 1.45%), Tennessee (40, 1.75%), Texas (381, 16.70%) and Virginia (5, 0.22%). The retrospective study population was comprised of US samples and samples collected outside the US (Spain). Each sample was tested at one of three clinical sites located in Minneapolis, MN; Louisville, KY; or Baltimore, MD using the Access anti-HBc IgM assay and commercially available anti-HBc IgM assays.

The Access anti-HBc IgM results for the prospective population for all clinical trial sites combined by age group and gender are summarized in the table below. Samples were considered nonreactive if S/CO was < 1.00 and reactive if S/CO was ≥ 1.00.

Distribution of Access anti-HBc IgM Reactive and Nonreactive Results Among the Prospective Cohort by Age Group and Sex

Age Range (Years)	Sex	Access anti-HBc IgM				Total
		Nonreactive		Reactive		
		N	%	N	%	
9 - 12	Female	6	0.26	0	0.00	6
	Male	11	0.48	0	0.00	11
13 - 18	Female	34	1.49	0	0.00	34
	Male	24	1.05	0	0.00	24
19 - 21	Female	73	3.20	0	0.00	73
	Male	28	1.23	0	0.00	28
22 - 29	Female	301	13.20	0	0.00	301
	Male	108	4.73	0	0.00	108
30 - 39	Female	276	12.10	0	0.00	276
	Male	129	5.66	0	0.00	129

Age Range (Years)	Sex	Access anti-HBc IgM				Total
		Nonreactive		Reactive		
		N	%	N	%	
40 - 49	Female	190	8.33	0	0.00	190
	Male	150	6.58	1	0.04	151
50 - 59	Female	253	11.09	0	0.00	253
	Male	225	9.86	1	0.04	226
60 - 69	Female	153	6.71	0	0.00	153
	Male	178	7.80	2	0.09	180
70 - 79	Female	52	2.28	0	0.00	52
	Male	55	2.41	0	0.00	55
80 - 89	Female	9	0.39	0	0.00	9
	Male	16	0.70	0	0.00	16
90+	Female	1	0.04	0	0.00	1
	Male	5	0.22	0	0.00	5
Total		**2,277**	**99.82**	**4**	**0.18**	**2,281**

Results by Specimen Classification

The HBV classification was determined by serological assessment for all prospective and retrospective specimens (n = 2,537) based on the reactivity patterns of six (6) HBV serological marker results (HBsAg, HBeAg, anti-HBc IgM, anti-HBc Total, anti-HBe, and anti-HBs) as illustrated in the table below. This testing was performed using FDA approved assays following the manufacturers' instructions for use.

Classification	HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc Total	Anti-HBe	Anti-HBs
Acute	+	+	+	+	-/+	-
	+	+	-/+	-	-	-
	+	-	-	-	-	-
	+	+	eq	+	-/+	-
	+	-	+	+	-	-
	+	-	eq	+	+	-
Acute (late)	+	-	+	+	+	-/+
Chronic	+	+	+	+	+	+
	+	-	-	+	+	-/+
	+	-	-	+	eq	-
	+	-	-	+	-	-/+
	+	+	+	+	-	+
	+	+	-	+	-	-/+

Classification	HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc Total	Anti-HBe	Anti-HBs
	+	+	-	+	+	-
Early Recovery	-	-	-	+	-/+	-
	-	-	+	+	-	-/+
	-	-	+	+	+	-/+
Recovery	-	-	-	-/+	+	+
	-	-	-	+	+	eq
Recovered or Immune due to Natural Infection	-	-	-	+	-	+, eq
HBV Vaccine Response	-	-	-	-	-	+
Possible HBV Vaccination Response	-	-	-	-	-	eq
Not Previously Infected	-	-	-	-	-	-
Not Interpretable	-	+	-	+	-	+
	-	-	-	-	+	-
	-	+	-	+	+	-
	-	+	-	-	-	-, eq, +

Notes: The symbols (+) and (-) refer to a final status of reactive and nonreactive, respectively. The symbol eq refers to equivocal.

Due to volume limitations, two (2) samples were classified with four serological markers (HBsAg, anti-HBc IgM, anti-HBc T and anti-HBs) as “susceptible” and “recovered or immune due to natural infection”.

Comparison of Results

All samples were tested by the Access anti-HBc IgM assay and anti-HBc IgM comparator assays with a final sample status determined by the Composite Reference Method (CRM) for Access anti-HBc IgM as outlined in table below.

Composite Reference Method (CRM) Status

Reference anti-HBc IgM	Comparator 1	Comparator 2	CRM Status
Reactive	Reactive	Reactive	Reactive
Reactive	Reactive	Nonreactive	Reactive
Reactive	Nonreactive	Reactive	Reactive
Reactive	Nonreactive	Nonreactive	Nonreactive
Equivocal	Reactive	Reactive	Reactive
Equivocal	Reactive	Nonreactive	Indeterminate*
Equivocal	Nonreactive	Reactive	Indeterminate*

Reference anti-HBc IgM	Comparator 1	Comparator 2	CRM Status
Equivocal	Nonreactive	Nonreactive	Nonreactive
Nonreactive	Not tested	Not tested	Nonreactive

*Final indeterminate results were excluded from analysis

Comparison of Results by HBV Classification Category

Access anti-HBc IgM results for each HBV classification were compared with the final interpretation from the CRM anti-HBc IgM assays in the table above.

The positive and negative percent agreement (PPA and NPA) between the Access anti-HBc IgM assay results and CRM status from the reference and comparator anti-HBc IgM assays for the prospective population based on HBV classification is summarized in the following table.

HBV Classification	Total	PPA		NPA	
		% (n/N)	95% CI	% (n/N)	95% CI
Acute	4	100.00 (2/2)	34.2 - 100.0	100.0 (2/2)	34.2 -100.0
Chronic	20	N/A (0/0)	N/A	95.00 (19/20)	76.4 - 99.1
Early Recovery	70	25.00 (1/4)**	4.6 - 69.9	100.0 (66/66)	94.5 - 100.0
HBV Vaccine Response	769	N/A (0/0)	N/A	100.0 (769/769)	99.5 - 100.0
Not Previously Infected	1,098	N/A (0/0)	N/A	100.0 (1,098/1,098)	99.7 - 100.0
Possible HBV Vaccination Response	73	N/A (0/0)	N/A	100.0 (73/73)	95.0 - 100.0
Recovered or Immune due to Natural Infection	121*	N/A (0/0)	N/A	100.0 (121/121)	96.9 - 100.0
Recovery	121	N/A (0/0)	N/A	100.0 (121/121)	96.9 - 100.0
Susceptible	1*	N/A (0/0)	N/A	100.0 (1/1)	20.7 - 100.0
Not Interpretable	4	N/A (0/0)	N/A	100.0 (4/4)	51.0 - 100.0
Total	**2,281**	**50.00 (3/6)**	**18.8 - 81.2**	**99.96 (2,274/2,275)**	**99.8 - 100.0**

*One subject classified with four-marker classification.

** All 4-four early recovery samples produced signals very close to the cutoff values of Access anti-HBc IgM and the reference and comparator anti-HBc IgM assays; two of the discordant samples produced results in concordance with the Access anti-HBc IgM upon re-testing (re-test results not used in performance calculations).

The PPA between the Access anti-HBc IgM assay results and CRM status from the reference and comparator anti-HBc IgM assays for the retrospective population, based on HBV classification, is summarized in the following table.

HBV Classification	Total	PPA	
		% (n/N)	95% CI
Acute	52	98.08 (51/52)	89.9 - 99.7
Acute (Late)	85	100.0 (85/85)	95.7 - 100.0

HBV Classification	Total	PPA	
		% (n/N)	95% CI
Chronic	5	100.0 (5/5)	56.6 - 100.0
Early Recovery	52	90.38 (47/52)	79.4 - 95.8
Not Interpretable	7	42.86 (3/7)	15.8 - 75.0
Total	**201**	**95.02 (191/201)**	**91.1 - 97.3**

Comparison of Results for Pregnant Women

One hundred and seventy-one (171) serum samples were prospectively collected from an increased risk and/or signs and symptoms U.S. pregnant population.

The NPA between the Access anti-HBc IgM assay results and CRM status from the reference and comparator anti-HBc IgM assays for the pregnant women population by HBV classification is summarized in the following table. This population included 21 pregnant subjects of pediatric age (range 18 - 21 years). There were 71 subjects in the first trimester, 67 subjects in the second trimester and 33 subjects in the third trimester in this study.

HBV Classification	Total	NPA	
		% (n/N)	95% CI
Chronic	1	100.0 (1/1)	20.65-100.0
HBV Vaccine Response	77	100.0 (77/77)	95.25-100.0
Not Previously Infected	82	100.0 (82/82)	95.52-100.0
Possible HBV Vaccination Response	9	100.0 (9/9)	70.09-100.0
Recovery	2	100.0 (2/2)	34.24-100.0
Total	**171**	**100.0 (171/171)**	**97.80-100.0**

Because no anti-HBc IgM positive samples were identified in the pregnant cohort, a spiking study was conducted to evaluate the results when pregnant samples are tested with the Access anti-HBc IgM assay. A total of thirty-one pregnant and control adult serum samples were spiked with a unique native anti-HBc IgM positive sample. The results showed that there were no significant differences between spiked pregnant samples versus control samples indicating that the Access anti-HBc IgM assay detects anti-HBc IgM comparably between pregnant and adult populations.

Comparison of Results for Pediatric Population

One hundred and fifty-four (155) prospective and eight (8) retrospective specimens were collected with risk factors and/or signs and symptoms from a pediatric (non-pregnant) population (9-21 years). The PPA and NPA between the Access anti-HBc IgM assay results and CRM status from the reference and comparator anti-HBc IgM assays for the prospective pediatric population, based on HBV classification, is summarized in the following table.

HBV Classification	Total	PPA		NPA	
		% (n/N)	95% CI	% (n/N)	95% CI
Early Recovery	1	0.00 (0/1)	0.0-79.3	N/A	N/A
HBV Vaccine Response	53	N/A	N/A	100.0 (53/53)	93.2-100.0
Not Previously Infected	95	N/A	N/A	100.0 (95/95)	96.1-100.0

HBV Classification	Total	PPA		NPA	
		% (n/N)	95% CI	% (n/N)	95% CI
Possible HBV Vaccination Response	3	N/A	N/A	100.0 (3/3)	43.9-100.0
Recovered or Immune due to Natural Infection	2	N/A	N/A	100.0 (2/2)	34.2-100.0
Not Interpretable	1	N/A	N/A	100.0 (1/1)	20.7-100.0
Total	**155**	**0.00 (0/1)**	**0.0-79.3**	**100.0 (154/154)**	**97.6-100.0**

The PPA between the Access anti-HBc IgM assay results and CRM status from the reference and comparator anti-HBc IgM assays for the pediatric retrospective population, based on HBV classification, is summarized in the following table.

HBV Classification	Total	PPA	
		% (n/N)	95% CI
Acute	2	100.0 (2/2)	34.2 - 100.0
Acute (Late)	2	100.0 (2/2)	34.2 - 100.0
Early Recovery	4	100.0 (4/4)	51.0 - 100.0
Total	**8**	**100.0 (8/8)**	**67.6 - 100.0**

Because few anti-HBc IgM positives were identified in the pediatric cohort, a spiking study was conducted to evaluate the results when pediatric samples are tested with the Access anti-HBc IgM assay. A total of thirty-one pediatric (age 3 – 21 years) and control adult serum samples were spiked with a unique native anti- HBc IgM positive sample. The results showed that there were no significant differences in results of spiked pediatric samples versus control samples indicating the Access anti-HBc IgM assay detects anti-HBc IgM comparably between pediatric and adult populations.

SEROCONVERSION

Commercially available patient seroconversion panels were tested using the Access anti-HBc IgM assay and a reference assay to determine the seroconversion sensitivity of the assay. Equivalent detection with no difference in bleed number was observed in 3 of the 6 panels and earlier detection by the Access anti-HBc IgM assay was observed in 3 panels. The results are summarized in the table below.

Panel ID	First anti-HBc IgM Positive Result from Initial Draw Date		Access anti-HBc IgM assay vs Reference assay
	Access anti-HBc IgM assay (days)	Reference assay (days)	Difference in bleed number for the first reactive bleed*
HBV-6281	43	43	0
HBV-9092	85	92	-1
HBV-9093	49	49	0
HBV-001	36	43	-1

Panel ID	First anti-HBc IgM Positive Result from Initial Draw Date		Access anti-HBc IgM assay vs Reference assay
	Access anti-HBc IgM assay (days)	Reference assay (days)	Difference in bleed number for the first reactive bleed*
HBV-002	59	59	0
HBV-004	71	76	-1

*The difference in bleed number is compared to the reference assay. For example, -1 indicates that the reference assay required 1 additional bleed before reactivity was determined compared to the Access anti-HBc IgM assay.

IMPRECISION

The imprecision of the Access anti-HBc IgM assay was evaluated in a study based on CLSI EP05-A3 guidance.[19] The study design included two test runs per day over a minimum of 20 test days. A ten-member panel of serum (S1-S4) and plasma (P1-P4) patient samples, and the Access anti-HBc IgM QC1 and QC2 were assayed in each run in triplicate. Three lots of Access anti-HBc IgM reagent and and two lots of Access anti-HBc IgM calibrator were tested on three DxI 9000 Access Immunoassay Analyzers for the study. The results for each lot are summarized in the following table.

			Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Between-Instrument		Within-Laboratory (Overall)	
Sample	N	Mean (S/CO)	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV
QC1	2,160	0.01	0.000	N/A	0.001	N/A	0.001	N/A	0.001	N/A	0.000	N/A	0.002	N/A
QC2	2,160	2.84	0.062	2.2	0.052	1.8	0.039	1.4	0.047	1.7	0.022	0.8	0.104	3.7
S1	2,160	0.01	0.000	N/A	0.000	N/A	0.000	N/A	0.001	N/A	0.000	N/A	0.001	N/A
S2	2,160	0.73	0.018	2.5	0.018	2.5	0.008	1.1	0.018	2.5	0.006	0.9	0.033	4.5
S3	2,160	1.13	0.028	2.5	0.033	2.9	0.015	1.3	0.036	3.1	0.010	0.9	0.059	5.2
S4	2,160	4.45	0.102	2.3	0.097	2.2	0.063	1.4	0.092	2.1	0.034	0.8	0.183	4.1
P1	2,160	0.01	0.001	N/A	0.000	N/A	0.000	N/A	0.001	N/A	0.000	N/A	0.001	N/A
P2	2,160	0.71	0.019	2.7	0.015	2.1	0.014	2.0	0.018	2.5	0.004	0.5	0.034	4.7
P3	2,160	1.08	0.030	2.8	0.018	1.7	0.031	2.9	0.038	3.6	0.008	0.8	0.061	5.7
P4	2,160	3.84	0.099	2.6	0.054	1.4	0.085	2.2	0.110	2.9	0.047	1.2	0.185	4.8

Note: %CV are not meaningful when S/CO approaches zero. Results are noted as N/A.

Reproducibility

A 5-day reproducibility study was performed on the DxI 9000 Access Immunoassay Analyzer based on CLSI EP05-A3 guidance.[19] A twelve-member panel of patient samples, including serum (S1-S6) and plasma (P1-P6) samples, were assayed at three clinical sites, using one lot of Access anti-HBc IgM reagent kit, on three instruments (one instrument per site). Each panel member was assayed in replicates of three at two separate times per day. The results are summarized in the following table.

			Repeatability (Within-Run)		Between-Run		Between-Day		Between-Site		Reproducibility	
Sample	N	Mean (S/CO)	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV
S1	90	0.01	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A
S2	90	0.32	0.009	2.7	0.003	1.0	0.000	0.0	0.011	3.6	0.015	4.5
S3	90	0.55	0.015	2.7	0.015	2.8	0.000	0.0	0.017	3.0	0.027	4.9
S4	90	0.76	0.018	2.4	0.009	1.2	0.003	0.4	0.024	3.2	0.032	4.2
S5	90	1.19	0.030	2.5	0.027	2.2	0.000	0.0	0.032	2.6	0.051	4.3
S6	90	4.52	0.108	2.4	0.068	1.5	0.049	1.1	0.087	1.9	0.162	3.6
P1	90	0.01	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A
P2	90	0.33	0.008	2.5	0.007	2.0	0.004	1.1	0.009	2.7	0.014	4.3
P3	90	0.53	0.013	2.6	0.012	2.2	0.000	0.0	0.017	3.2	0.025	4.7
P4	90	0.74	0.019	2.6	0.013	1.8	0.000	0.0	0.021	2.8	0.031	4.2
P5	90	1.12	0.024	2.1	0.029	2.6	0.000	0.0	0.035	3.1	0.052	4.6
P6	90	3.95	0.097	2.5	0.081	2.1	0.042	1.1	0.073	1.8	0.152	3.8

Note: %CV are not meaningful when S/CO approaches zero. Results are noted as N/A.

INTERFERING SUBSTANCES

The Access anti-HBc IgM assay was evaluated for interference consistent with CLSI document EP07 3rd Edition.[20] Testing was performed using one negative and two reactive samples (one low positive and one moderate positive) with substances at concentrations indicated. Of the compounds tested, none were found to cause interference using the highest test concentrations indicated in the following table.

Potential Interferent	Highest Concentration Added
Hemoglobin	1,000 mg/dL
Total Protein	15 g/dL
Bilirubin Conjugated	40 mg/dL
Bilirubin Unconjugated	40 mg/dL
Biotin	3,510 ng/mL
Cholesterol	400 mg/dL
Triglycerides (Intralipid)	37 mmol/L (3,854 mg/dL)
Aspirin (acetylsalicylic acid)	167 µmol/L
Salicylic acid	207 µmol/L
Acetaminophen (paracetamol)	1,030 µmol/L
Ibuprofen	1,060 µmol/L
Atorvastatin	1.34 µmol/L
Lisinopril	0.607 µmol/L
Levothyroxine	0.552 µmol/L

Potential Interferent	Highest Concentration Added
Metformin	92.9 µmol/L
Amlodipine	0.183 µmol/L
Omeprazole	24.3 µmol/L
Sertraline	3.03 µmol/L

CROSS REACTIVITY

Cross-reactivity was evaluated by testing samples for potentially cross-reacting conditions. No cross-reactivity was observed. The results are summarized in the following table.

Category	Number of samples tested	Number of Reactive samples	Number of Nonreactive samples
Epstein-Barr virus (EBV IgM or VCA IgG)	10	0	10
Cytomegalovirus (CMV)	10	0	10
Herpes simplex virus (HSV 1/2)	10	0	10
Human immunodeficiency virus (HIV)	10	0	10
Hepatitis A virus (HAV)	10	0	10
Hepatitis C virus (HCV)	10	0	10
Hepatitis E virus (HEV)	10	0	10
Alcoholic liver disease	10	0	10
Primary biliary cirrhosis	10	0	10
Flavivirus (Zika)	10	0	10
Flavivirus (Dengue)	10	0	10
Flavivirus (West Nile)	10	0	10
Influenza post-vaccination	10	0	10
HAMA	10	0	10
Anti-nuclear antibody (ANA)	10	0	10
Rheumatoid Factor	10	0	10
Systemic lupus erythematosus (SLE)	10	0	10
Multiple myeloma	10	0	10
Pregnancy multipara	10	0	10
Pregnancy first trimester	10	0	10
Pregnancy second trimester	10	0	10
Pregnancy third trimester	10	0	10
Syphilis	10	0	10
Toxoplasmosis	10	0	10
Transplant recipient	10	0	10
Dialysis patients	10	0	10

Category	Number of samples tested	Number of Reactive samples	Number of Nonreactive samples
Hemophiliac / Clotting factor deficiency	10	0	10
anti-E. coli (including E. coli urinary infection)	10	0	10
Rubella	10	0	10
Varicella Zoster Virus (VZV)	10	0	10
Measles	10	0	10
Mumps	10	0	10

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

Initial release.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
+(1) 800-854-3633
www.beckmancoulter.com