ACCESS
Immunoassay Systems

ACCESS HBsAg CALIBRATOR
Hepatitis B Virus Surface Antigen

REF C39423

Instructions For Use

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

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 HBsAg Calibrator is intended to calibrate the Access HBsAg and Access HBsAg Confirmatory assays for the *in vitro* qualitative detection and confirmation of hepatitis B surface antigen (HBsAg) in human serum and plasma using the DxI 9000 Access Immunoassay Analyzer.

For additional information, refer to the Access HBsAg and Access HBsAg Confirmatory reagent Instructions for Use.

SUMMARY AND EXPLANATION

The Access HBsAg Calibrator is used to establish calibration (determine the cut-off value) for the Access HBsAg and Access HBsAg Confirmatory assays. By comparing the light intensity generated by a sample to the cut-off value, the presence or absence of hepatitis B virus surface antigen in the sample is determined.

TRACEABILITY

The analyte in the Access HBsAg Calibrator is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

Value assignment was established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. The assigned value has been embedded in the calibrator bar code. The values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

REAGENTS

CONTENTS

Access HBsAg Calibrator
Ref. No. C39423: C1, 2.0 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Do not use kit reagents beyond the expiration date.
- Vials are stable at 2 to 10°C for 180 days after initial use.
- Signs of possible deterioration are quality control values out of range.

C1	TRIS buffer with HBs antigen (human), protein (bovine), < 0.1% sodium azide, and 0.5% ProClin* 300.
Calibration Card	2

*ProClin is a trademark of LANXESS Corp.

Card 1 is for use with the Access HBsAg (REF C39422)

Card 2 is for use with the Access HBsAg Confirmatory (REF C39425)

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,[1] 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 regulations and guidelines.
- This product contains materials of human and animal origins and should be considered as potentially capable of transmitting infectious diseases.
- 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

ACCESS HBsAg CALIBRATOR

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.

reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

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

CALIBRATION

CALIBRATION INFORMATION

The Access HBsAg Calibrator is used for both the Access HBsAg and the Access HBsAg Confirmatory assays. It is provided at one level: positive for HBsAg.

Run the calibrator in duplicate. Assay calibration data is valid for 56 days.

TESTING PROCEDURE(S)

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

PROCEDURAL NOTES

LIMITATIONS

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

Initial release.

SYMBOLS KEY

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

REFERENCES

1. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication, 6th ed., June 2020.

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ACCESS Immunoassay Systems
Instructions For Use

ACCESS HBsAg CONFIRMATORY
Hepatitis B Virus Surface Antigen
REF C39425

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI 9000 Access Immunoassay Analyzers

PRINCIPLE

CAUTION

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INTENDED USE

The Access HBsAg Confirmatory assay is a paramagnetic particle, chemiluminescent immunoassay for the *in vitro* qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human pediatric (7 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)] that have been found to be repeatedly reactive in the Access HBsAg assay using the DxI 9000 Access Immunoassay Analyzer.

The Access HBsAg Confirmatory assay is for use on the DxI 9000 Access Immunoassay System only.

SUMMARY AND EXPLANATION

Hepatitis B, caused by hepatitis B virus infection, 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, which surrounds the hepatitis B core antigen (HBc Ag).[2] 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] HBV is replicated via an RNA intermediate. Mutations are common in HBV which could impact the detection of HBV infection, disease progression, and treatment.[4] Ten genotypes of HBV, designated A through J based on the divergency in the nucleotide sequences, are associated with clinical outcomes.[5] In addition, multiple serologic subtypes of HBV have been identified based on the heterogeneity in antigenic determinants of HBsAg.[5]

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]

HBsAg can be detected in the blood as early as 1 to 2 weeks after infection.[6] HBV infection is either symptomatic or, more commonly, asymptomatic, particularly among children. For adults, most HBV infections are self-limited. Less than 5% of

infections do not resolve and subsequently develop into persistent infections, which are either symptomatic or asymptomatic.[3,7] If the infection is symptomatic, the clinical manifestations of acute disease are typically expressed 2-3 months following viral exposure and can last for 2-4 months. Symptoms may include fatigue, poor appetite, nausea, vomiting, abdominal pain, low-grade fever, jaundice, dark urine and light stool color.[6] The presence of HBsAg for at least six months indicates chronic hepatitis B infection.[8] Since chronic infections are dynamic and may transition through different clinical phases, testing for alanine aminotransferase (ALT), HBV DNA and HBV antigens is useful in management of chronic HBV infection.[5,6,8,9]

HBsAg is a serologic marker of acute and chronic HBV infection. The presence of HBsAg indicates a person is infectious regardless of whether the infection is acute or chronic.[6] Identifying infected individuals helps prevent transmission. The World Health Organization (WHO) recommends testing all donors (blood, plasma, and organs) for hepatitis B to avoid transmission.[1] In addition, HBsAg testing should be part of antenatal screening programs to avoid transmitting hepatitis B from mother to fetus.[1,6]

The Access HBsAg Confirmatory assay uses specific antibody neutralization to confirm the presence of HBsAg in samples that repeatedly test reactive with the HBsAg assay. An HBsAg neutralization test is the most common test method provided by manufacturers for confirming repeat reactive HBsAg results. The Access HBsAg Confirmatory assay verifies that all HBsAg reactive results are caused by HBsAg in the sample and not by non-specific interference. A reactive sample is considered positive for HBsAg if the confirmatory test neutralizes the sample, which confirms the presence of HBsAg.[1,6]

METHODOLOGY

Assay Type: one-step neutralization

The Access HBsAg Confirmatory assay uses the principle of neutralization by an excess of HBsAg-specific antibodies (neutralization reagent) to confirm the presence of HBsAg in samples found repeatedly reactive in the Access HBsAg assay. The confirmatory assay reports percent neutralization (suppression) of a control assay with a neutralization reagent.

Paramagnetic particles coated with monoclonal HBsAg antibodies, alkaline phosphatase coupled to polyclonal HBsAg antibodies and sample are added to two separate reaction vessels, one containing neutralization reagent, and the other containing diluent (control). If HBsAg is present in the sample, the HBsAg specific antibodies of the neutralization reagent bind to the antigenic determinants which can no longer bind to the antibodies of the solid phase and conjugate. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. A chemiluminescent substrate is added to each vessel and light generated by the reaction is measured with a luminometer. Both neutralization and diluent (control) tests are automatically run on the system. Percent neutralization is determined by comparison of the light generated with the neutralization reagent to that of the diluent (control).

SPECIMEN

SPECIMEN STORAGE AND 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	6	30
Plasma	Lithium Heparin Lithium Heparin separator tube Dipotassium (K_2) EDTA Tripotassium (K_3) EDTA Sodium Citrate			Do not thaw samples more than 5 times.

Specimen	Type	20 to 25°C (hours)	2 to 8°C (days)	-20°C or colder (days)
	Acid Citrate Dextrose (ACD) Citrate Phosphate Dextrose (CPD)			

SPECIMEN COLLECTION AND PREPARATION

Blood Specimen

1. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[10,11] To minimize the effect of preanalytical factors observe the following recommendations for handling and processing blood samples:[10]

   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. Avoid assaying lipemic or hemolyzed samples.

REAGENTS

CONTENTS

Access HBsAg Confirmatory Reagent Pack
Ref. No. C39425: 100 determinations (sufficient for 100 patient samples), 2 packs, 50/pack

Well	Contents	Ingredients
R1a:	3.30 mL	Paramagnetic particles coated with streptavidin coupled to biotinylated monoclonal (mouse) HBsAg antibodies in TRIS buffer, surfactant, protein (bovine), < 0.1% sodium azide and 0.1% ProClin* 300.
R1b:	2.10 mL	MES buffer, protein (bovine), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	3.20 mL	MES buffer, polyclonal (caprine) HBsAg antibody alkaline phosphatase conjugate, surfactant, protein (bovine, caprine and mouse), < 0.1% sodium azide and 0.1% ProClin 300.

Well	Contents	Ingredients
R1d:	1.20 mL	MES buffer, polyclonal (equine) HBsAg antibody and protein (bovine), < 0.1% sodium azide and 0.1% ProClin 300.

*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,[12] 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 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

HBsAg 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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%
HBsAg 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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
HBsAg 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.

		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%
HBsAg Blocking Reagent (Compartment R1d)	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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] nd 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access HBsAg Reagent Pack
   200 determinations, 2 packs, 100 tests/pack
   Ref. No. C39422
2. Access HBsAg Calibrator
   Provided as one positive level for HBsAg
   Ref. No. C39423
3. Access HBsAg QC
   Provided as three vials each of HBsAg positive control and HBsAg negative control
   Ref. No. C39424
4. Lumi-Phos PRO
   Ref. No. B96000
5. 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 point is required for all tests. 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.

Note: The Access HBsAg Calibrator kit contains two calibration cards. There is one calibration card for the Access HBsAg assay and one calibration card for the Access HBsAg Confirmatory assay.

Use the Access HBsAg Calibrator and Access HBsAg Confirmatory calibration card to calibrate the Access HBsAg Confirmatory assay. The Access HBsAg Confirmatory S/CO ratio is determined by the active calibration.

QUALITY CONTROL

Refer to Quality Control Instructions for Use.

Quality control materials are essential for monitoring the system performance. Quality controls should be run individually at least once every 24 hours when the assay is being performed.[13] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

The Access HBsAg QC is used with the Access HBsAg and Access HBsAg Confirmatory assays. Run the Access HBsAg QC2 positive control at least once every 24 hours while performing the Access HBsAg Confirmatory assay.

Quality Control	Control (S/CO)	Percent Neutralization (% NT)
QC2	Reactive See value card in Access HBsAg QC kit	≥ 50% See value card in Access HBsAg QC kit

Note: Do not dilute Access HBsAg QC.

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 contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
4. The Access HBsAg Confirmatory assay requires 90 µL of sample in addition to the sample container and sample dead volumes. Refer to the appropriate system manuals and/or help system for minimum sample volume required.
5. The Access HBsAg Confirmatory assay uses antigen neutralization. Samples that repeatedly test reactive with the Access HBsAg assay are incubated with and without neutralizing reagent. Samples that contain neutralized HBsAg produce lower RLU than non-neutralized samples. The percent (%) neutralization is used to confirm the presence of HBsAg in reactive samples.
6. The Access HBsAg Confirmatory assay percent (%) neutralization is calculated by the system using the following formula: [1.00 - (HBsBk* RLU/HBsCt** RLU)] x 100

   *HBsBk (HBsAg Blocking assay/Neutralizing assay) is the reaction vessel that contains the neutralization reagent.
   **HBsCt (HBsAg Control assay/Specimen Control assay) is the reaction vessel that contains the diluent (control).
7. Dilute the sample with wash buffer if the S/CO ratio is greater than or equal to 1.00 and the % of neutralization is less than 50%. See the Reporting Results section for dilution instructions. **Do not enter the dilution factor in the Dilution Factor Field because this is a qualitative assay.**
8. Test the diluted sample with the Access HBsAg Confirmatory assay.

LIMITATIONS

1. The Access HBsAg Confirmatory assay is approved for *in vitro* diagnostic use only.
2. This product is for use on the DxI 9000 Access Immunoassay Analyzer only.
3. Do not use kits or components beyond the stated expiration date.
4. The Access HBsAg Confirmatory assay is limited to the confirmation of the presence of hepatitis B virus surface in human serum, including 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).
5. 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.
6. The performance of this test has not been established in cord blood, neonates or children younger than 7 years of age.
7. 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.[14,15] These interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

8. 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.[16,17]
9. A nonreactive HBsAg Confirmatory test result does not exclude the possibility of exposure to or infection with hepatitis B virus. The Access HBsAg 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.
10. Do not use specimens with obvious microbial contamination.
11. Avoid assaying grossly hemolyzed or lipemic samples.
12. The Access HBsAg Confirmatory assay does not demonstrate any "hook" effect up to an HBsAg concentration of 2.5 mg/mL.

RESULTS INTERPRETATION

Access HBsAg Confirmatory test results are determined automatically by the system software. The presence of HBsAg in a reactive sample is confirmed if the calculations performed by the DxI 9000 Access Immunoassay Analyzer show a Signal/Cut-off (S/CO) ratio that is ≥ 1.00, and a percent neutralization (%NT) that is ≥ 50%.

Samples with a S/CO ratio that is ≥ 1.00 and < 50% neutralization may be diluted up to 1:62,500, as indicated in the following table. Review the test results on the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions about reviewing sample results.

REPORTING RESULTS

Neat Sample

Result (S/CO)	Percent Neutralization (%NT)	Interpretation	Reporting Instructions	Retest procedure
< 1.00	Not applicable	Nonreactive	Nonreactive for the presence of HBsAg	No retest required
≥ 1.00	≥ 50%	Confirmed	Confirmed positive for the presence of HBsAg	No retest required
≥ 1.00	< 50%	Not Confirmed	Not Applicable. Follow retest procedure	Dilute sample 1:250 and run in Access HBsAg Confirmatory Assay

Prepare 1:250 dilution of sample

1. Dilute a sample 1:250 with UniCel DxI Wash Buffer II if the neat sample was not confirmed:
   a. Dispense 10 µL of sample and 90 µL UniCel DxI Wash Buffer II into test tubes. Thoroughly mix the test tubes to be sure the sample and Wash Buffer II are sufficiently combined (1:10 intermediate dilution).
   b. Dispense 10 µL of the 1:10 intermediate dilution and 240 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly

mix the test tubes to be sure the 1:10 intermediate dilution and Wash Buffer II are sufficiently combined (1:250 final dilution).

2. Run on the DxI 9000 Access Immunoassay Analyzer. **Do not enter the dilution factor in the Dilution Factor Field because this is a qualitative assay. A sample comment may be added, as appropriate.** Always assign a unique Sample ID to patient samples that were diluted offline prior to analysis.

Result (S/CO)	Percent Neutralization (%NT)	Interpretation	Reporting Instructions	Retest procedure
< 1.00	Not applicable	Nonreactive	Report negative for the presence of HBsAg	No retest required
≥ 1.00	≥ 50%	Confirmed	Report confirmed positive for the presence of HBsAg	No retest required
≥ 1.00	< 50%	Not Confirmed	Not Applicable. Follow retest procedure	Dilute sample 1:62,500 and run in Access HBsAg Confirmatory assay

Prepare 1:62,500 dilution of sample (use dilution method 1 or 2 below)

1. Dilute a sample 1:62,500 with UniCel DxI Wash Buffer II if the 1:250 dilution of sample was not confirmed, using previously prepared 1:250 dilution.

   a. Dispense 10 µL of the 1:250 final dilution and 90 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly mix the test tubes to be sure the 1:250 final dilution and Wash Buffer II are sufficiently combined (1:2,500 intermediate dilution).

   b. Dispense 10 µL of the 1:2,500 intermediate dilution and 240 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly mix the test tubes to be sure the 1:2,500 intermediate dilution and Wash Buffer II are sufficiently combined (1:62,500 final dilution).

2. Dilute a sample 1:62,500 with UniCel DxI Wash Buffer II if the 1:250 dilution of sample was not confirmed, preparing a fresh dilution.

   a. Dispense 10 µL of sample and 90 µL UniCel DxI Wash Buffer II into test tubes. Thoroughly mix the test tubes to be sure the sample and Wash Buffer II are sufficiently combined (1:10 intermediate dilution).

   b. Dispense 10 µL of the 1:10 intermediate dilution and 240 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly mix the test tubes to be sure the 1:10 intermediate dilution and Wash Buffer II are sufficiently combined (1:250 intermediate dilution).

   c. Dispense 10 µL of the 1:250 intermediate dilution and 90 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly mix the test tubes to be sure the 1:250 intermediate dilution and Wash Buffer II are sufficiently combined (1:2,500 intermediate dilution).

   d. Dispense 10 µL of the 1:2,500 intermediate dilution and 240 µL UniCel DxI Wash Buffer II into a clean test tube. Thoroughly mix the test tubes to be sure the 1:2,500 intermediate dilution and Wash Buffer II are sufficiently combined (1:62,500 final dilution).

3. Run on the DxI 9000 Access Immunoassay Analyzer. **Do not enter the dilution factor in the Dilution Factor Field because this is a qualitative assay. A sample comment may be added, as appropriate.** Always assign a unique Sample ID to patient samples that were diluted offline prior to analysis.

Result (S/CO)	Percent Neutralization (%NT)	Interpretation	Reporting Instructions	Retest procedure
< 1.00	Not applicable	Nonreactive	Report negative for the presence of HBsAg	No retest required
≥ 1.00	≥ 50%	Confirmed	Report confirmed positive for the presence of HBsAg	No retest required

After dilution 1:62,500, results ⩾ 1.00 S/CO and < 50% NT are considered highly unlikely. If the situation is observed, it is recommended to repeat the confirmatory test.

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

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

CLINICAL PERFORMANCE EVALUATION

The Access HBsAg Confirmatory assay performance was evaluated during the Access HBsAg clinical study. The table below describes the number of specimens repeatedly reactive with Access HBsAg and the number of specimens confirmed with the Access HBsAg Confirmatory assay on the Access DxI 9000 immunoassay analyzer.

	Adult IR/S&S (N)	Adult Pre-characterized acute/chronically ill (N)	Pregnant IR/S&S (N)	Pregnant Low risk (N)	Pediatric IR/S&S (N)	Total (N)
Overall Population	2,474	464	183	312	181	3,614
Access HBsAg – Repeat Reactive	25	397	1	0	0	423
Access HBsAg Confirmatory Reactive	23	396	1	0	0	420 [a]

IR/S&S = Increased Risk and/or Signs & Symptoms

[a] The three samples found repeat reactive using Access HBsAg were found nonreactive using both Access HBsAg Confirmatory and the Reference HBsAg assay.

Clinical Agreement by HBV Classification

Results of testing using the Access HBsAg Confirmatory assay for all testing sites by HBV classification are presented for the increased risk and signs and symptoms population (including retrospective HBsAg positive samples from acute or chronic patients with HBV infection) in the following table. The agreement between the Access HBsAg Confirmatory assay and an FDA approved reference HBsAg Confirmatory assay for each HBV specimen classification is listed in the following table.

Classification	Access HBsAg Assay with Confirmatory			Reference HBsAg Assay with Confirmatory		
	Repeat Reactive (N)	Confirmed Reactive (%)	95% CI	Repeat Reactive (N)	Confirmed Reactive (%)	95% CI
Early Acute	3	100.0% (3/3)	43.9-100.0	3	100.0% (3/3)	43.9-100.0

Classification	Access HBsAg Assay with Confirmatory			Reference HBsAg Assay with Confirmatory		
	Repeat Reactive (N)	Confirmed Reactive (%)	95% CI	Repeat Reactive (N)	Confirmed Reactive (%)	95% CI
Acute	17	100.0% (17/17)	81.6-100.0	17	100.0% (17/17)	81.6-100.0
Chronic	393	100.0% (393/393)	99.0-100.0	393	100.0% (393/393)	99.0-100.0
Immune due to Natural Infection	2	100.0% (2/2)	34.2-100.0	0	N/A	N/A
Immune due to HBV Vaccination	1	0.0% (0/1)	N/A	0	N/A	N/A
Uninterpretable	5	60.0% (3/5)[a]	23.1-88.2	0	N/A	N/A
Missing Classification	2	100.0% (2/2)	34.2-100.0	2	100.0% (2/2)	34.2-100.0
Total	**423**	**99.3% (420/423)**	**97.9-99.8**	**415**	**100.0% (415/415)**	**99.1-100.0**

[a] Three of five samples were confirmed reactive using the Access HBsAg Confirmatory assay: available seroprofile information for these samples may indicate a low level of HBsAg that was not detected by the reference assay.

The following table provides a sub analysis of samples where the Access HBsAg initial results were reactive (1.00 ≤ S/CO < 100.00) and required repeat testing and confirmation.

HBV Classification	Reference HBsAg Assay with Confirmation				Total
	Reactive		Nonreactive		
	Access HBsAg with confirmatory				
	Reactive	Nonreactive	Reactive	Nonreactive	
Early Acute	1	0	0	0	1
Acute	1	0	0	0	1
Chronic	13	0	0	0	13
Immune due to Natural Infection	0	0	2	0	2
Immune due to HBV Vaccination	0	0	0	1	1
Uninterpretable	0	0	3[a]	2	5
Missing Classification	1	0	0	0	1
Total	**16**	**0**	**5**	**3**	**24**

[a] Samples were confirmed reactive using Access HBsAg Confirmatory: available seroprofile information for these samples may indicate a low level of HBsAg that was not detected by the reference assay.

Results in Pregnant Population

495 serum samples were prospectively collected from a U.S. pregnant screening population, including 183 with at-risk factors for hepatitis and/or signs and symptoms of hepatitis. Samples were tested using the Access HBsAg assay and the reference HBsAg qualitative and reference HBsAg confirmatory assay to provide a final interpretation. The results of HBsAg testing at all sites combined are presented in the following table.

Pregnancy Cohort	Trimester	Reference HBsAg with Reference HBsAg Confirmatory assay result				Total (N)
		Reactive		Nonreactive		
		Access HBsAg		Access HBsAg		
		Repeat Reactive (N)	Nonreactive (N)	Repeat Reactive (N)	Nonreactive (N)	
Pregnancy Screening (IR/S&S*) n = 183	First	1	0	0	74	75
	Second	0	0	0	71	71
	Third	0	0	0	37	37
Pregnancy Screening (Low Risk) n = 312	First	0	0	0	127	127
	Second	0	0	0	109	109
	Third	0	0	0	76	76
Total	**Total**	**1**	**0**	**0**	**494**	**495**

*IR/S&S = Increased Risk and/or Signs & Symptoms

The one sample that was found repeat reactive using Access HBsAg confirmed positive with Access HBsAg Confirmatory. The positive and negative percent agreement between Access HBsAg and the reference HBsAg assay final interpretations for the pregnant women population is summarized in the following table. This population included 60 pregnant subjects of pediatric age (range 18 –21 years).

Pregnancy Cohort	PPA		NPA	
Trimester	**% (n/N)**	**95% CI**	**% (n/N)**	**95% CI**
First	100.0 (1/1)	20.7-100.0	100.0 (201/201)	98.1-100.0
Second	N/A	N/A	100.0 (180/180)	97.9-100.0
Third	N/A	N/A	100.0 (113/113)	96.7-100.0
Total	**100.0 (1/1)**	**20.7-100.0**	**100.0 (494/494)**	**99.2-100.0**

Analytical comparison between pregnant and adult non-pregnant samples spiked with HBsAg

Thirty (30) samples from HBsAg negative pregnant patients were tested to determine if they provided equivalent results to HBsAg negative adult human serum pool samples (controls) when samples were spiked with the same individual HBsAg positive donor. These 30 samples included two specimens from women in the 1st trimester, 16 from the 2nd trimester, and 12 from the 3rd trimester of pregnancy.

Samples were spiked at target HBsAg values between 2.00 and 4.00 S/CO and tested with the Access HBsAg assay in replicates of five. All samples were confirmed reactive with the Access HBsAg Confirmatory assay.

Comparison of Results for Pediatric Population

181 serum samples were prospectively collected from an at-risk and signs and symptoms U.S. pediatric non-pregnant population. Samples were tested using the Access HBsAg assay and the reference HBsAg qualitative and reference HBsAg confirmatory assay to provide a final interpretation. No reactive samples were found with Access HBsAg; therefore, no Access HBsAg Confirmatory testing was required. Negative percent agreement between the Access HBsAg assay and the reference HBsAg assay in pediatric samples was calculated. The results are summarized in the following table.

PPA		NPA	
% (n/N)	95% CI	% (n/N)	95% CI
N/A	N/A	100.0 (181/181)	97.9-100.0

Analytical comparison between pediatrics and adult samples spiked with HBsAg

Thirty (30) samples from HBsAg negative pediatric patients aged 7 to 20 were tested to determine if they provided equivalent results to HBsAg negative adult human serum pool samples (controls) when samples were spiked with the same individual HBsAg positive donor.

Samples were spiked at a target HBsAg value between 2.00 and 4.00 S/CO and tested with the Access HBsAg assay in replicates of five. All samples were confirmed reactive with Access HBsAg Confirmatory assay.

Distribution of Access HBsAg Confirmatory Results

The table below summarizes the Access HBsAg initial reactive results and corresponding Access HBsAg Confirmatory results:

Access HBsAg Result (S/CO)	Access HBsAg Initial Reactive N, (% of Total)	Access HBsAg Confirmatory N, (% of Initial Reactive confirmed within S/CO range)
1.00 - <10.00	19 (4.5)	16 (84.2)[a]
10.00 - <50.00	2 (0.5)	2 (100.0)
50.00 - <100.00	3 (0.7)	3 (100.0)
100.00 - <500.00	9 (2.1)	9 (100.0)
500.00 - <1000.00	9 (2.1)	9 (100.0)
1000.00 - <2000.00	22 (5.2)	22 (100.0)
2000.00 - <3000.00	36 (8.5)	36 (100.0)
3000.00 - <5000.00	69 (16.3)	69 (100.0)
5000.0 - <7000.00	73 (17.3)	73 (100.0)
7000.00 - <9000.00	120 (28.4)	120 (100.0)
9000.0+	61 (14.4)	61 (100.0)
Total	**423 (100.0)**	**420 (99.3)**

[a] Three samples <10.00 S/CO were found nonreactive using Access HBsAg Confirmatory; all three were also found nonreactive using the reference HBsAg assay.

ANALYTICAL SENSITIVITY

The Access HBsAg Confirmatory assay was designed to have an analytical sensitivity of less than or equal to 0.03 IU/mL using the WHO Third International Standard (I.S.) for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2), NIBSC code: 12/226.

To examine the analytical sensitivity of the Access HBsAg Confirmatory assay on the DxI 9000 Access Immunoassay Analyzer, a series of dilutions were prepared using the WHO Third I.S. with target HBsAg sample concentrations in the range of 0.01 – 0.1 IU/mL. The dilutions were assayed using three reagent and three calibrator lots on two DxI 9000 Access Immunoassay Analyzers over three days. The analytical sensitivity for each reagent/calibrator lot combination was determined using a linear fit regression of the S/CO of the control results versus the WHO I.S. concentrations (IU/mL). When the %NT was ≥ 50%, the point estimate of the WHO I.S. concentration corresponding to S/CO = 1.00 in the control assay and the 95% CI were used in the analytical sensitivity estimation. The maximum overall analytical sensitivity results determined in this study are summarized in the following table:

Standard	Analytical Sensitivity
WHO 3rd International standard NIBSC 12/226	0.019 IU/mL (95% CI: 0.018 – 0.020 IU/mL)

INTERFERING SUBSTANCES

The Access HBsAg Confirmatory assay was evaluated for interference consistent with CLSI document EP07 3rd Edition.[18] Testing was performed using two weakly reactive samples (one low positive and one moderate positive) at concentrations indicated. Of the compounds tested, none were found to cause significant interference using the highest test concentrations indicated in the following table.

Substance	Highest concentration added
Hemoglobin	1000 mg/dL
Total Protein	15 g/dL
Bilirubin conjugated	43 mg/dL
Bilirubin unconjugated	43 mg/dL
Intralipid	38.54 mg/mL (37 mmol/L)
Biotin	3510 ng/mL
Aspirin (acetylsalicylic acid)	167 µmol/L
Salicylic Acid	207 µmol/L
Acetaminophen (paracetamol)	1030 µmol/L
Ibuprofen	1060 µmol/L
Atorvastatin	1.34 µmol/L
Lisinopril	0.607 µmol/L
Levothyroxine	0.552 µmol/L
Metformin	92.9 µmol/L
Amlodipine	0.183 µmol/L
Omeprazole	862.26 µmol/L
Sertraline	3.03 µmol/L

CROSS REACTIVITY

Cross-reactivity was evaluated by spiking the potentially cross reactant sample with human HBsAg antigen. For preparation of bacterial and antigen samples, negative samples were spiked with bacteria or antigens and then spiked with human HBsAg antigen prior to evaluation. All samples were confirmed for the presence of HBsAg using the Access HBsAg Confirmatory assay, and a commercially available HBsAg Confirmatory assay (not shown). The results are summarized in the following table.

Category	Number of HBsAg samples tested	Number of samples confirmed for the presence of HBsAg	Number of samples not confirmed for the presence of HBsAg
Epstein-Barr Virus	2	2	0
Cytomegalovirus (CMV)	2	2	0
Herpes Simplex Virus (HSV 1/2)	2	2	0
Human Immunodeficiency Virus (HIV)	2	2	0

Category	Number of HBsAg samples tested	Number of samples confirmed for the presence of HBsAg	Number of samples not confirmed for the presence of HBsAg
Hepatitis A Virus (HAV)	2	2	0
Hepatitis C Virus (anti-HCV)	2	2	0
Hepatitis E Virus (HEV)	2	2	0
Alcoholic Liver Disease	2	2	0
Primary Biliary Cirrhosis	2	2	0
Flavivirus (Zika)	2	2	0
Flavivirus (Dengue)	2	2	0
Toxoplasmosis (Anti-Toxo IgG)	2	2	0
Syphilis	2	2	0
Flavivirus (West nile)	2	2	0
S.aureus	2	2	0
P.aeruginosa	2	2	0
E.coli	2	2	0
Influenza Post Vaccination	2	2	0
HAMA	2	2	0
Anti-Nuclear Antibody (ANA)	2	2	0
Rheumatoid Factor	2	2	0
Systemic Lupus Erythematosus (SLE)	2	2	0
Pregnancy Multipara	2	2	0
Pregnancy First Trimester	2	2	0
Pregnancy Second Trimester	2	2	0
Pregnancy Third Trimester	2	2	0
Transplant / Transplant recipient	2	2	0
Dialysis Patients	2	2	0
Hemophiliac / Clotting factor	2	2	0

In addition, Access HBsAg and Access HBsAg Confirmatory assays evaluated for 11 other disease categories by spiking 10 negative samples with antigen for each of the following categories: Cytomegalovirus (CMV), Epstein-Bar virus (EBV), Hepatitis A Virus (HAV),Hepatitis C Virus (HCV), Hepatitis E Virus (HEV), Human Immunodeficiency Virus (HIV), Herpes Simplex virus (HSV1/2), Rubella, Varicella Zoster Virus (VZV), Toxoplasmosis, and Syphilis. No cross reactivity was observed.

GENOTYPE DETECTION

Genotype detection of the Access HBsAg Confirmatory assay was evaluated using 2 commercially available genotype panels (22 samples) and two patient samples containing genotypes A through H. A total of 24 panel members (A (4), B (3), C (4), D (4), E (2), F (3), G (1) and H (3)) were tested using the Access HBsAg and Access HBsAg Confirmatory assays. All genotypes were reactive by the Access HBsAg assay and confirmed reactive for the presence of HBsAg using the Access HBsAg Confirmatory assay.

SUBTYPE DETECTION

A total of 9 commercially available subtypes were tested using the Access HBsAg and Access HBsAg Confirmatory assays: adw2 (1), adw4 (1), adr (1), ayw1 (1), ayw2 (1), ayw3 (2), ayw4 (1) and ayr (1). All subtypes were found reactive by the Access HBsAg assay and confirmed reactive for the presence of HBsAg using the Access HBsAg Confirmatory assay.

MUTANT DETECTION

A total of 30 (10 native and 20 recombinant) HBsAg mutant samples, containing defined mutations between amino acid 100 and 170 were tested using three different batches of Access HBsAg Confirmatory reagent kits. The mutant samples were diluted and tested at concentrations below 3.00 S/CO using a commercially available HBsAg assay. These mutant samples represent the most common HBsAg mutants reported in literature.[19, 20, 21, 22, 23] All 30 HBsAg mutant samples were confirmed reactive with an S/CO greater than or equal to 1.00 S/CO (1.28 S/CO to 4.38 S/CO) and a percent inhibition greater than or equal to 50% (78% to 94%).

Mutant Type	Mutation
Native	Q129R+G130N, T126N, G130R+S132Y, P120S+F134I, T126A, Q129H+D144A, T118A (2), Y137F, M133L
Recombinant	P142S, S143L, G145R, C137W, P142S+G145R, 122NT, C124R, D144A, F134H, G130N, K122T, L109I, M133T, P127T, Y161F, Q129H, T123N, T126S, T131A, Y100C

ADDITIONAL INFORMATION

SYMBOLS KEY

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

REVISION HISTORY

Revision A

Initial release.

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ACCESS
Immunoassay Systems

ACCESS HBsAg
Hepatitis B Virus Surface Antigen

REF C39422

Instructions For Use

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

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 HBsAg assay is a paramagnetic particle, chemiluminescent immunoassay for the *in vitro* qualitative detection of hepatitis B surface antigen (HBsAg) in human pediatric (7 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 HBsAg assay is used for the laboratory diagnosis of acute or chronic hepatitis B virus (HBV) infection in individuals with signs and symptoms of hepatitis or at risk for hepatitis B virus infection, when used in conjunction with other serological and clinical information. The assay may also be used to screen for HBV infection in pregnant women to identify neonates who are at risk of acquiring hepatitis B during the perinatal period.

The Access HBsAg assay is for use on the DxI 9000 Access Immunoassay Analyzer only.

This assay has not been approved for use in the screening of blood, plasma, or tissue donors or cadaveric specimens.

SUMMARY AND EXPLANATION

Hepatitis B, caused by HBV infection, 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, which surrounds the hepatitis B core antigen (HBc Ag).[2] 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] HBV is replicated via an RNA intermediate.

Mutations are common in HBV which could impact the detection of HBV infection, disease progression, and treatment.[4] Ten genotypes of HBV, designated A through J based on the divergency in the nucleotide sequences, are associated with clinical outcomes.[5] In addition, multiple serologic subtypes of HBV have been identified based on the heterogeneity in antigenic

determinants of HBsAg.[5]

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]

HBsAg can be detected in the blood as early as 1 to 2 weeks after infection.[6] HBV infection is either symptomatic or, more commonly, asymptomatic, particularly among children. For adults, most HBV infections are self-limited. Less than 5% of infections do not resolve and subsequently develop into persistent infections, which are either symptomatic or asymptomatic.[3,7] If the infection is symptomatic, the clinical manifestations of acute disease are typically expressed 2-3 months following viral exposure and can last for 2-4 months. Symptoms may include fatigue, poor appetite, nausea, vomiting, abdominal pain, low-grade fever, jaundice, dark urine and light stool color.[6] The presence of HBsAg for at least six months indicates chronic hepatitis B infection.[8] Since chronic infections are dynamic and may transition through different clinical phases, testing for alanine aminotransferase (ALT), HBV DNA and HBV antigens is useful in management of chronic HBV infection.[5,6,8,9]

HBsAg is a serologic marker of acute and chronic HBV infection. The presence of HBsAg indicates a person is infectious regardless of whether the infection is acute or chronic.[6] Identifying infected individuals helps prevent transmission. The World Health Organization (WHO) recommends testing all donors (blood, plasma, and organs) for hepatitis B to avoid transmission.[1] In addition, HBsAg testing should be part of antenatal screening programs to avoid transmitting hepatitis B from mother to fetus.[1,6]

According to the testing algorithm validated during the Access HBsAg clinical trial, specimens found nonreactive upon initial testing using the Access HBsAg assay are considered negative for HBsAg. Specimens found initially reactive with S/CO values ≥ 100.00 should be reported as positive for the presence of HBsAg and no additional retest or confirmatory testing is required. Specimens found initially reactive with S/CO values ≥ 1.00 and < 100.00 must be retested in duplicate to determine whether they are repeatedly reactive. If found repeatedly reactive using the Access HBsAg assay, the result should be confirmed using the Access HBsAg Confirmatory assay (REF C39425), which uses specific antibody neutralization to confirm the presence of HBsAg. A reactive sample is considered positive for HBsAg if the confirmatory test neutralizes the sample, which confirms the presence of HBsAg.[1,6]

METHODOLOGY

Assay type: one-step, sandwich

The Access HBsAg assay is a one-step enzyme immunoassay. Paramagnetic particles coated with monoclonal HBsAg antibodies, alkaline phosphatase coupled to polyclonal HBsAg antibodies and sample are added to a reaction vessel. HBsAg present in the patient sample is bound by the anti-HBs coated on the paramagnetic particles and the anti-HBs-alkaline phosphatase conjugate. After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. A chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is compared to the cut-off value defined during calibration on the instrument. 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	6	30
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)			Do not thaw samples more than 5 times.

SPECIMEN COLLECTION AND PREPARATION

Blood Specimen

1. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[10,11] To minimize the effect of preanalytical factors observe the following recommendations for handling and processing blood samples:[10]

   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. Avoid assaying lipemic or hemolyzed samples.

REAGENTS

CONTENTS

Access HBsAg Reagent Pack
Ref. No. C39422, 200 determinations, 2 packs, 100 tests/pack

Well	Contents	Ingredients
R1a:	3.30 mL	Paramagnetic particles coated with streptavidin coupled to biotinylated monoclonal (mouse) HBsAg antibodies in TRIS buffer, surfactant, protein (bovine), < 0.1% sodium azide and 0.1% ProClin* 300.
R1b:	3.00 mL	MES buffer, protein (bovine), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	3.20 mL	MES buffer, polyclonal (caprine) HBsAg antibody alkaline phosphatase conjugate, surfactant, protein (bovine, caprine and mouse), < 0.1% sodium azide and 0.1% ProClin 300.

*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,[12] 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 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 section: 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

HBsAg 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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%
HBsAg 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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
HBsAg 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.
	reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access HBsAg Calibrator
   Provided as one positive level for HBsAg
   Ref. No. C39423
2. Quality Control (QC) materials: Access HBsAg QC

   Provided as three vials each of HBsAg positive control and HBsAg negative control
   Ref. No. C39424
3. Access HBsAg Confirmatory Reagent Kit

   100 determinations (sufficient for 100 patient samples), 2 packs, 50/pack
   Ref. No. C39425
4. Lumi-Phos PRO
   Ref. No. B96000
5. 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. 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.

Note: The Access HBsAg Calibrator kit contains two calibration cards. There is one calibration card for the Access HBsAg assay and one calibration card for the Access HBsAg Confirmatory assay.

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.[13] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

Include commercially available quality control materials that cover at least 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 45 µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. The system default unit of measure for sample results is the Signal/Cut-off (S/CO) ratio.

LIMITATIONS

1. The Access HBsAg assay is approved 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 HBsAg assay is limited to the detection of surface antigen to hepatitis B virus in human serum, including 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)).
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 children younger than 7 years of age.
8. 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.[14,15] These interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
9. 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.[16,17]
10. A nonreactive HBsAg test result does not exclude the possibility of exposure to or infection with hepatitis B virus. The Access HBsAg 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.
11. A reactive result obtained with the Access HBsAg assay result does not exclude co-infection by other hepatitis viruses.
12. Do not use specimens with obvious microbial contamination.
13. Persons who have been vaccinated with a recombinant hepatitis B vaccine within the last month could potentially test positive for hepatitis B surface antigen in the absence of infection.[18, 19]
14. The calculated Signal/Cut-off (S/CO) values for HBsAg 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.
16. The Access HBsAg assay does not demonstrate any “hook” effect up to an HBsAg concentration of 2.5 mg/mL.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. Results (Signal/Cut-off (S/CO)) are reported to be “reactive” or “nonreactive” as a function of their relationship with the “cut-off” (signal “greater than or equal to” or “less than” the cut-off 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.

The high positive cut-off value (≥ 100.00 S/CO of HBsAg) for not performing repeat testing on Access HBsAg or confirmation testing on the Access HBsAg Confirmatory was determined using a population of reactive and nonreactive clinical samples. The cut-off was validated by comparing results from samples with initial S/CO values ≥ 100.00 with the final result using the Access HBsAg Confirmatory assay, as well as the final interpretation using commercially available reference HBsAg Qualitative and reference HBsAg Confirmatory assays. In this study, 399/423 (94.3%) samples initially reactive with Access HBsAg assay fell into the ≥ 100.00 S/CO range and 100.0% (399/399) were confirmed positive using the Access HBsAg Confirmatory assay. Positive percent agreement (PPA) of the Access HBsAg assay with the HBsAg reference assay final interpretation was also 100.0% with a lower bound 2-sided 95% score confidence interval of 99.0%.

REPORTING RESULTS

Initial Results

Result (S/CO)	Interpretation	Reporting Instructions	Retest procedure
< 1.00	Nonreactive	Report result as nonreactive for HBsAg	No retest required
≥ 1.00 to < 100.00	Reactive	Report as initial reactive for HBsAg	Retest in duplicate
≥ 100.00	Reactive	Report as positive (reactive) for the presence of HBsAg	No retest or additional confirmatory testing required

Duplicate Retest Results

Result (S/CO)	Interpretation	Reporting Instructions	Retest procedure
Both results < 1.00	Nonreactive	Report result as nonreactive for HBsAg	No retest required
One or both results ≥ 1.00	Reactive	Report result as repeat reactive for HBsAg	Confirm repeat reactive result in Access HBsAg Confirmatory assay (REF C39425). Samples that are confirmed using the neutralization reagent can be considered positive for the presence of HBsAg.

PERFORMANCE CHARACTERISTICS

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 Access DxI 9000 Analyzer to evaluate the ability of the Access HBsAg assay to detect HBsAg in specimens from the intended use population. The overall study population included 3,614 specimens, consisting of 3,150 serum samples that were prospectively collected, and an additional 464 pre-characterized retrospective samples (serum and plasma) collected from patients with acute and chronic HBV infection. Of the 3,150 specimens prospectively collected, 2,474 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. 495 prospective specimens were from the pregnant screening population, of which 183 also were increased risk and/or showed signs and symptoms of hepatitis. Specimens from the pregnant population included 202 from the first trimester, 180 from the second trimester, and 113 from the third trimester. In addition, 181 prospective specimens were collected from the pediatric population with increased risk and/or signs & symptoms. The table below summarizes the number of specimens in each population.

	Adult (non-pregnant) (n=2,938)		Pregnant (n=495)		Pediatric (non-pregnant)
	IR/S&S*	Pre-characterized acute/chronic HBV infection	IR/S&S	Low risk	IR/S&S
Prospective (n=3,150)	2,474	N/A	183	312	181
Retrospective (n=464)	N/A	464	N/A	N/A	N/A
Total	**3,614**				

*IR/S&S = Increased Risk and/or Signs & Symptoms

Expected Results

The prospective study population was 62.6% White, 26.7% Black or African American, 1.4% Asian, 1.4% American Indian or Alaska Native, 0.2% Native Hawaiian or other Pacific Islander, and 7.7% from unknown/other or unwilling to answer. 28.9% of the prospective study population was of Hispanic ethnicity. The majority of patients were female (65% female and 35% male). Patients in the prospective population were from the following states: Arizona (586, 18.6%), California (1, 0.0%), Connecticut (148, 4.7%), Florida (457, 14.5%), Georgia (118, 3.7%), Indiana (90, 2.9%), North Carolina (5, 0.2%), New Jersey (90, 2.9%), New York (518, 16.4%), Ohio (54, 1.7%), Pennsylvania (201, 6.4%), South Carolina (95, 3.0%), Tennessee, (225,

7.1%), Texas (392, 12.4%), and Virginia (170, 5.4%). Each sample was tested at one of three clinical sites located in Minneapolis, MN; Louisville, KY; or Baltimore, MD using the Access HBsAg assay and a commercially available HBsAg assay.

The Access HBsAg 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 upon initial testing and repeat reactive if S/CO was ≥ 1.00 during initial testing and 2 of 3 replicates were ≥ 1.00 S/CO after duplicate testing.

Distribution of Access HBsAg Assay Repeat Reactive and Nonreactive Results Among the Prospective Cohort by Age Group and Gender

Age Range (years)	Sex	Access HBsAg				Total
		Repeat Reactive		Nonreactive		
		N	%	N	%	
7-12*	Female	0	0.00	14	0.44	14
	Male	0	0.00	19	0.60	19
13-18	Female	0	0.00	43	1.37	43
	Male	0	0.00	24	0.76	24
19-21	Female	0	0.00	109	3.46	109
	Male	0	0.00	32	1.02	32
22-29	Female	1	0.03	519	16.48	520
	Male	0	0.00	126	4.00	126
30-39	Female	2	0.06	478	15.17	480
	Male	2	0.06	165	5.24	167
40-49	Female	3	0.10	257	8.16	260
	Male	3	0.10	171	5.43	174
50-59	Female	4	0.13	336	10.67	340
	Male	5	0.16	265	8.41	270
60-69	Female	0	0.00	207	6.57	207
	Male	5	0.16	198	6.29	203
70-79	Female	0	0.00	62	1.97	62
	Male	1	0.03	63	2.00	64
80-89	Female	0	0.00	13	0.41	13
	Male	0	0.00	17	0.54	17
90+	Female	0	0.00	1	0.03	1
	Male	0	0.00	5	0.16	5
Total		**26**	**0.83**	**3,124**	**99.17**	**3,150**

*Four (4) subjects outside of the pediatric intended use population of 7-21 years are included in this analysis

Results by Specimen Classification

The HBV classification was determined by serological assessment for all prospective and retrospective specimens (n=3,614) based on the reactivity patterns of four HBV serological marker results (HBsAg, anti-HBc IgM, anti-HBc Total and anti-HBs). This testing was performed using FDA approved serologic assays from one manufacturer following that manufacturer's instructions for use. Fifteen (15) unique reference marker patterns were observed in the study and are shown in the table below.

	HBsAg[a]	Anti-HBc IgM	Anti-HBc Total	Anti-HBs
Early Acute	+	-	-	-
Acute	+	+	+	-
	+	I	+	-
Recovering Acute/Undetectable HBsAg	-	+	+	-
Recovering Acute	-	+	+	+
Chronic	+	-	+	+
	+	-	+	-
Immune due to Natural Infection	-	-	+	+
Immune due to HBV Vaccination	-	-	-	+
Susceptible	-	-	-	-
Uninterpretable	-	-	+	-
	-	-	+	I
	-	-	-	I
	-	I	+	+
	-	+	-	-

I = Indeterminate
+ = Positive/reactive
- = Negative/nonreactive
[a] HBsAg results indicated as + were repeatedly reactive and confirmed by neutralization as required by the manufacturer's Instructions for Use.

Comparison of Results

Access HBsAg results for each classification were compared with the final interpretation from the same reference HBsAg qualitative assay with confirmatory (if needed) used for classification in the table above. The results are described in the table below.

Comparison of Access HBsAg assay versus HBsAg Reference Assay – All Populations by HBV Classification

Cohort	Reference HBsAg Assay with Reference HBsAg Confirmatory assay result				Total		
	Reactive		Nonreactive				
	Access HBsAg						
	Nonreactive (N)	Repeat Reactive (N)	Nonreactive (N)	Repeat Reactive (N)	Prospective (N)	Retrospective (N)	Total (N)
Early Acute	0	3	0	0	3	0	3
Acute	0	17	0	0	2	15	17
Recovering Acute	0	0	4	0	4	0	4
Chronic	1[a]	393	0	0	18	376	394
Immune due to Natural Infection	0	0	264	2	226	40	266
Immune due to HBV Vaccination	0	0	1,164	1[b]	1,163	2	1,165
Susceptible	0	0	1,552	0	1,549	3	1,552
Uninterpretable	0	0	203	5[c]	183	25	208
Missing Classification	1[d]	2	2	0	2	3	5
Total	**2**	**415**	**3,189**	**8**	**3,150**	**464**	**3,614**

[a] Sample tested PCR negative by certificate of analysis.

[b] Sample tested nonreactive using the Access HBsAg Confirmatory assay.

[c] Three of five samples were confirmed reactive using the Access HBsAg Confirmatory assay: available seroprofile information for these samples may indicate a low level of HBsAg that was not detected by the reference assay. The remaining two samples were found nonreactive with Access HBsAg Confirmatory.

[d] Sample tested PCR negative by supplementary testing and by certificate of analysis.

The following table provides a sub analysis of samples where the Access HBsAg initial results were reactive (1.00 ≤ S/CO < 100.00) and required repeat testing and confirmation.

HBV Classification	Reference HBsAg with Reference HBsAg Confirmatory assay result				Total
	Reactive		Nonreactive		
	Access HBsAg				
	Initial Reactive (N)	Nonreactive (N)	Initial Reactive (N)	Nonreactive (N)	
Early Acute	1	0	0	0	1
Acute	1	0	0	0	1
Chronic	13	0	0	0	13
Immune due to Natural Infection	0	0	2	0	2
Immune due to HBV Vaccination	0	0	1[a]	0	1
Uninterpretable	0	0	5[b]	0	5
Missing Classification	1	0	0	0	1
Total	**16**	**0**	**8**	**0**	**24**

[a] Sample tested nonreactive using the Access HBsAg Confirmatory assay.

[b] Three of five samples were confirmed reactive using the Access HBsAg Confirmatory assay: available seroprofile information for these samples may indicate a low level of HBsAg that was not detected by the reference assay. The remaining two samples were found nonreactive with Access HBsAg Confirmatory.

The following table provides a sub analysis of results from high positive (reactive) samples where no repeat or confirmatory testing was required using the high positive cut-off (S/CO ≥ 100.00).

HBV Classification	Reference HBsAg with Reference HBsAg Confirmatory assay result				Total
	Reactive		Nonreactive		
	Access HBsAg				
	Reactive	Nonreactive	Reactive	Nonreactive	
Early Acute	2	0	0	0	2
Acute	16	0	0	0	16
Chronic	380	0	0	0	380
Missing Classification	1	0	0	0	1
Total	**399**	**0**	**0**	**0**	**399**

Percent Agreement by HBV Classification

The positive percent agreement (PPA) for repeat reactive samples and negative percent agreement (NPA) between the Access HBsAg assay results and the reference HBsAg assay final interpretation for each HBV specimen classification is summarized in the following table for all populations.

Cohort	PPA		NPA	
	% (n/N)	95% CI	% (n/N)	95% CI
Early Acute	100.0% (3/3)	43.9-100.0%	N/A	N/A
Acute	100.0% (17/17)	81.6-100.0%	N/A	N/A
Recovering Acute	N/A	N/A	100.0% (4/4)	51.0-100.0%
Chronic	99.7% (393/394)[a]	98.6-100.0%	N/A	N/A
Immune due to Natural Infection	N/A	N/A	99.2% (264/266)	97.3-99.8%
Immune due to HBV Vaccination	N/A	N/A	99.9% (1,164/1,165)[b]	99.5-100.0%
Susceptible	N/A	N/A	100.0% (1,552/1,552)	99.8-100.0%
Uninterpretable	N/A	N/A	97.6% (203/208)[c]	94.5-99.0%
Missing Classification	66.7% (2/3)[d]	20.8-93.9%	100.0% (2/2)	34.2-100.0%
Total	**99.5% (415/417)**	**98.3-99.9%**	**99.7% (3,189/3,197)**	**99.5-99.9%**

[a] The one sample found nonreactive using Access HBsAg also tested PCR negative by certificate of analysis.

[b] The one sample found repeat reactive using Access HBsAg was found nonreactive using Access HBsAg Confirmatory.

[c] Of five samples found repeat reactive with Access HBsAg, three were confirmed reactive using Access HBsAg Confirmatory: available seroprofile information for these samples may indicate a low level of HBsAg that was not detected by the reference assay. The remaining two samples tested nonreactive with Access HBsAg Confirmatory.

[d] The one sample found nonreactive using Access HBsAg was found PCR negative by supplementary testing and by certificate of analysis.

The positive percent agreement between the Access HBsAg assay repeat reactive results and the reference assay final interpretation for the prospective, retrospective and combined populations is summarized in the following table.

Cohort	PPA		NPA	
	% (n/N)	95% CI	% (n/N)	95% CI
Prospective	100.0 (23/23)	85.7-100.0	99.7 (3,189/3,197)	99.5-99.9
Retrospective	99.5 (392/394)[a]	98.2-99.9	N/A	N/A
Combined	**99.5 (415/417)**	**98.3-99.9**	**N/A**	**N/A**

[a] The two samples found nonreactive using Access HBsAg tested PCR negative by certificate of analysis or supplementary testing.

Comparison of Results for Pregnant Women by Trimester

495 serum samples were prospectively collected from a U.S. pregnant screening population, including 183 with at-risk factors for hepatitis and/or signs and symptoms of hepatitis. Samples were tested using the Access HBsAg assay and the reference HBsAg qualitative and reference HBsAg confirmatory assay to provide a final interpretation. The results of HBsAg testing at all sites combined are presented in the following table.

Pregnancy Cohort	Trimester	Reference HBsAg with Reference HBsAg Confirmatory assay result				Total (N)
		Reactive		Nonreactive		
		Access HBsAg				
		Repeat Reactive (N)	Nonreactive (N)	Repeat Reactive (N)	Nonreactive (N)	
Pregnancy Screening (IR/S&S*) n = 183	First	1	0	0	74	75
	Second	0	0	0	71	71
	Third	0	0	0	37	37
Pregnancy Screening (Low Risk) n = 312	First	0	0	0	127	127
	Second	0	0	0	109	109
	Third	0	0	0	76	76
Total	**Total**	**1**	**0**	**0**	**494**	**495**

*IR/S&S = Increased Risk and/or Signs & Symptoms

The positive percent agreement and negative percent agreement between the Access HBsAg assay results and the reference HBsAg assay final interpretation for the pregnant women population is summarized in the following table. This population included 60 pregnant subjects of pediatric age (range 18 –21 years).

Pregnancy Cohort	PPA		NPA	
Trimester	**% (n/N)**	**95% CI**	**% (n/N)**	**95% CI**
First	100.0 (1/1)	20.7-100.0	100.0 (201/201)	98.1-100.0
Second	N/A	N/A	100.0 (180/180)	97.9-100.0
Third	N/A	N/A	100.0 (113/113)	96.7-100.0
Total	**100.0 (1/1)**	**20.7-100.0**	**100.0 (494/494)**	**99.2-100.0**

Analytical comparison between pregnant and adult non-pregnant samples spiked with HBsAg

Thirty (30) samples from HBsAg negative pregnant patients were tested to determine if they provided equivalent results to HBsAg negative adult human serum pool samples (controls) when samples were spiked with the same individual HBsAg positive donor. These 30 samples included two specimens from women in the 1st trimester, 16 from the 2nd trimester, and 12 from the 3rd trimester of pregnancy.

Samples were spiked at target HBsAg values between 2.00 and 4.00 S/CO and tested with the Access HBsAg assay in replicates of five. The S/CO results for each pregnant patient sample were compared to the result obtained on each control sample and ranged from -6% to 13%. All samples were confirmed reactive with the Access HBsAg Confirmatory assay.

Comparison of Results for Pediatric Population

181 serum samples were prospectively collected from an at-risk and signs and symptoms U.S. pediatric non-pregnant population. Samples were tested using the Access HBsAg assay and the reference HBsAg qualitative and reference HBsAg confirmatory assay to provide a final interpretation. No positive samples were obtained and negative percent agreement between the Access HBsAg assay and the reference HBsAg assay in pediatric samples was calculated. The results are summarized in the following table.

PPA		NPA	
% (n/N)	**95% CI**	**% (n/N)**	**95% CI**
N/A	N/A	100.0 (181/181)	97.9-100.0

Analytical comparison between pediatrics and adult samples spiked with HBsAg

Thirty (30) samples from HBsAg negative pediatric patients aged 7 to 20 were tested to determine if they provided equivalent results to HBsAg negative adult human serum pool samples (controls) when samples were spiked with the same individual HBsAg positive donor.

Samples were spiked at a target HBsAg value between 2.00 and 4.00 S/CO and tested with the Access HBsAg assay in replicates of five. The S/CO results for each pediatric patient sample were compared to the result obtained on each control sample and ranged from -13% to 6%. All samples were confirmed reactive with Access HBsAg Confirmatory assay.

ANALYTICAL SENSITIVITY

The Access HBsAg assay was designed to have an analytical sensitivity of less than or equal to 0.03 IU/mL using the WHO Third International Standard (I.S.) for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2), NIBSC code: 12/226.

To examine the analytical sensitivity of the Access HBsAg assay on the DxI 9000 Access Immunoassay Analyzer, a series of dilutions was prepared using the WHO Third I.S. with target HBsAg sample concentrations in the range of 0.01 – 0.1 IU/mL. The dilutions were assayed using three reagent and three calibrator lots on two DxI 9000 Access Immunoassay Analyzers over three days. The analytical sensitivity for each reagent/calibrator lot combination was determined using a linear fit regression of the S/CO versus the WHO I.S. concentrations (IU/mL). The point estimate of the WHO I.S. concentration corresponding to S/CO = 1.00 and the 95% CI were used in the analytical sensitivity estimation. The maximum overall analytical sensitivity results determined in this study are summarized in the following table:

Standard	Analytical Sensitivity
WHO 3rd International standard NIBSC code: 12/226	0.025 IU/mL (95% CI: 0.024 – 0.026 IU/mL)

A limit of detection (LoD) study was conducted on the DxI 9000 Access Immunoassay Analyzer following methods described in CLSI guideline EP17-A2 using dilutions of the WHO 3rd International standard NIBSC code: 12/226. Five dilutions in serum and five dilutions in plasma were prepared ranging from 0.020 IU/mL to 0.024 IU/mL, and each dilution level was tested across three reagent lots.

The LoD was determined for serum and plasma for each reagent lot by modeling the relationship between the percent of replicates detected (S/CO ≥ 1.00) and sample concentration using the Probit approach to calculate the concentration corresponding to 95% probability of detection. The maximum observed LoD value across reagent lots was 0.024 IU/mL for both plasma and serum sample types.

SEROCONVERSION

30 commercially available patient seroconversion panels were tested using the Access HBsAg assay and a reference assay to determine the seroconversion sensitivity of the assay. The results are summarized in the following table.

Panel ID	HBsAg Positive Result from Initial Draw Date		Access HBsAg assay vs Reference HBsAg assay
	Access HBsAg assay (days)	Reference HBsAg assay (days)	Difference in bleed number*
SCP-HBV-002	16	16	0
SCP-HBV-004	18	18	0
SCP-HBV-006	13	13	0
HBV6273	14	14	0
HBV6275	7	7	0
HBV6277	33	33	0
HBV6278	12	8	+1
HBV6279	26	26	0
HBV6280	13	13	0
HBV6282	19	19	0
HBV6283	26	26	0
HBV6284	50	50	0
HBV6285	40	40	0
HBV6286	33	33	0
HBV6290	21	14	+2
HBV6291	27	27	0
HBV9099	16	16	0
HBV11000	21	21	0
HBV11001	44	44	0
HBV11003	142	142	0
HBV11007	34	34	0
HBV11011	103	103	0
HBV11012	18	18	0
HBV11017	40	40	0
HBV11029	35	35	0

Panel ID	HBsAg Positive Result from Initial Draw Date		Access HBsAg assay vs Reference HBsAg assay
	Access HBsAg assay (days)	Reference HBsAg assay (days)	Difference in bleed number*
HBV11052	37	37	0
HBV11056	33	33	0
PHM937	2	2	0
PHM939	11	3	+1
PHM941	7	7	0

*The difference in bleed number is compared to the reference assay. For example, +1 indicates that the reference assay required 1 less bleed before reactivity was determined compared to the Access HBsAg assay.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- ≤ 0.100 S/CO SD at concentrations of < 1.00 S/CO
- ≤ 10.0% CV at concentrations ≥ 1.00 S/CO

The imprecision of the Access HBsAg assay was evaluated in a study based on CLSI EP05-A3 guidance.[20] The study design included two test runs per day over 20 test days. A ten-member panel of serum (S1-S5), plasma (P1-P5) patient samples and one lot of QC (QC1-QC2) were assayed in each run with patient samples tested in triplicate. Three lots of Access HBsAg reagents, two lots of calibrators and two lots of QC were tested on one DxI 9000 Access Immunoassay Analyzer for the study. The results presented below are overall results.

Sample	Mean (S/CO)	N	Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory (Total)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC1	0.12	1,440	0.00	N/A	0.00	N/A	0.00	N/A	**0.01**	N/A
QC2	2.90	1,440	0.05	1.7	0.03	1.1	0.06	1.9	0.15	**5.3**
P1	0.12	1,440	0.00	N/A	0.00	N/A	0.00	N/A	**0.01**	N/A
P2	1.28	1,440	0.02	1.5	0.01	1.1	0.02	1.7	0.07	**5.3**
P3	3.18	1,440	0.05	1.5	0.05	1.7	0.05	1.4	0.17	**5.2**
P4	6.23	1,440	0.10	1.7	0.08	1.4	0.11	1.8	0.34	**5.4**
P5	156.97	1,440	2.63	1.7	1.97	1.3	2.42	1.5	10.88	**6.9**
S1	0.11	1,440	0.00	N/A	0.00	N/A	0.00	N/A	0.01	N/A
S2	1.23	1,440	0.02	1.5	0.01	1.1	0.02	1.7	**0.06**	5.1
S3	2.91	1,440	0.04	1.5	0.03	1.2	0.05	1.6	0.15	**5.1**
S4	4.51	1,440	0.07	1.6	0.05	1.1	0.08	1.7	0.23	**5.1**
S5	152.09	1,440	2.53	1.7	1.59	1.0	2.56	1.7	10.33	**6.8**

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.[20] A ten-member panel of patient samples, including serum (S1-S5) and plasma (P1-P5) samples were assayed at three external clinical sites using one lot of Access HBsAg reagent kits, on three instruments. Each panel member was assayed in replicates of three in two runs per day. The results are summarized in the following table.

			Between-Site		Between-Day		Between-Run		Repeatability (Within-Run)		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	0.11	0.02	N/A	0.00	N/A	0.00	N/A	0.01	N/A	0.02	N/A
S2	90	1.23	0.12	9.9	0.01	0.8	0.04	2.9	0.06	4.7	0.14	11.4
S3	90	2.94	0.32	10.8	0.05	1.8	0.07	2.2	0.04	1.5	0.33	11.2
S4	90	4.56	0.44	9.6	0.00	0.0	0.16	3.6	0.20	4.4	0.51	11.2
S5	90	155.93	19.45	12.5	5.48	3.5	4.29	2.8	2.29	1.5	20.79	13.3
P1	90	0.12	0.02	N/A	0.00	N/A	0.00	N/A	0.01	N/A	0.02	N/A
P2	90	1.28	0.13	10.3	0.00	0.0	0.02	1.7	0.03	2.1	0.14	10.7
P3	90	3.20	0.34	10.5	0.04	1.1	0.03	0.9	0.06	1.7	0.34	10.7
P4	90	6.34	0.57	9.0	0.09	1.4	0.07	1.1	0.11	1.8	0.59	9.3
P5	90	159.12	17.86	11.2	5.79	3.6	3.46	2.2	2.86	1.8	19.3	12.1

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

INTERFERING SUBSTANCES

The Access HBsAg assay was evaluated for interference consistent with CLSI document EP07 3rd Edition.[21] Testing was performed using one negative and two weakly reactive samples (one low positive and one moderate positive) at concentrations indicated. Of the compounds tested, none were found to cause interference using the highest test concentrations indicated in the following table.

Substance	Highest Concentration Added
Hemoglobin	1,000 mg/dL
Total Protein	15 g/dL

Substance	Highest Concentration Added
Bilirubin conjugated	43 mg/dL
Bilirubin unconjugated	43 mg/dL
Intralipid	38.54 mg/mL (37 mmol/L)
Biotin	3,510 ng/mL
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
Metformin	92.9 µmol/L
Amlodipine	0.183 µmol/L
Omeprazole	862.26 µmol/L
Sertraline	3.03 µmol/L

CROSS REACTIVITY

Cross-reactivity was evaluated by testing samples for potentially cross-reacting conditions. For preparation of bacterial and antigen samples, negative samples were spiked with bacteria and/or antigen prior to evaluation. 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 IgG)	10	0	10
Cytomegalovirus (CMV IgG and IgM)	10	0	10
Herpes Simplex Virus (HSV 1/2 IgG)	10	0	10
Human Immunodeficiency Virus (HIV)	10	0	10
Hepatitis A Virus (HAV)	10	0	10
Hepatitis C Virus (anti-HCV)	10	0	10
Hepatitis E Virus (HEV)	10	0	10
Alcoholic Liver Disease	10	0	10

Category	Number of samples tested	Number of Reactive samples	Number of Nonreactive samples
Primary Biliary Cirrhosis	10	0	10
Flavivirus (Zika)	11	0	11
Flavivirus (Dengue)	8	0	8
Flavivirus co-infected (Zika + Dengue)	2	0	2
Toxoplasmosis (Anti-Toxo IgG)	10	0	10
Syphilis	10	0	10
Flavivirus (West Nile)	10	0	10
S.aureus	10	0	10
P. aeruginosa	10	0	10
E.coli	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
Pregnancy Multipara	10	0	10
Pregnancy First Trimester	10	0	10
Pregnancy Second Trimester	10	0	10
Pregnancy Third Trimester	10	0	10
Transplant / Transplant recipient	10	0	10
Dialysis Patients	10	0	10
Hemophiliac / Clotting factor	10	0	10

In addition, Access HBsAg and Access HBsAg Confirmatory assays were evaluated for 11 other disease categories by spiking 10 negative samples with antigen for each of the following categories: Cytomegalovirus (CMV), Epstein-Bar virus (EBV), Hepatitis A Virus (HAV),Hepatitis C Virus (HCV), Hepatitis E Virus (HEV), Human Immunodeficiency Virus (HIV), Herpes Simplex virus (HSV1/2), Rubella, Varicella Zoster Virus (VZV), Toxoplasmosis, and Syphilis. No cross reactivity was observed.

GENOTYPE DETECTION

Genotype detection of the Access HBsAg assay was evaluated using 2 commercially available genotype panels (22 samples)

and two patient samples containing genotypes A through H. A total of 24 panel members (A (4), B (3), C (4), D (4), E (2), F (3), G (1) and H (3)) were tested using the Access HBsAg and Access HBsAg Confirmatory assays. All genotypes were reactive by the Access HBsAg assay (and confirmed reactive for the presence of HBsAg using the Access HBsAg Confirmatory assay).

SUBTYPE DETECTION

A total of 9 commercially available subtypes were tested using the Access HBsAg assay (adw2 (1), adw4 (1), adr (1), ayw1 (1), ayw2 (1), ayw3 (2), ayw4 (1) and ayr (1)). All subtypes were reactive by the Access HBsAg assay and confirmed reactive for the presence of HBsAg using the Access HBsAg Confirmatory assay.

MUTANT DETECTION

A total of 30 (10 native and 20 recombinant) HBsAg mutant samples, containing defined mutations between amino acid 100 and 170 were tested. The mutant samples were diluted and tested at concentrations below 3.00 S/CO using a commercially available HBsAg assay. These HBsAg mutant samples represent the most common HBsAg mutants reported in literature. [22, 23, 24, 25,26] All 30 HBsAg mutants were recognized using the Access HBsAg assay and a commercially available HBsAg assay (and confirmed reactive for the presence of HBsAg using the Access HBsAg Confirmatory assay).

Mutant Type	Mutation
Native	Q129R+G130N, T126N, G130R+S132Y, P120S+F134I, T126A, Q129H+D144A, T118A (2), Y137F, M133L
Recombinant	P142S, S143L, G145R, C137W, P142S+G145R, 122NT, C124R, D144A, F134H, G130N, K122T, L109I, M133T, P127T, Y161F, Q129H, T123N, T126S, T131A, Y100C

ADDITIONAL INFORMATION

SYMBOLS KEY

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

REVISION HISTORY

Revision A

Initial release.

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ACCESS Immunoassay Systems
Instructions For Use

ACCESS HBsAg QC
Hepatitis B Virus Surface Antigen
REF C39424

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

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 HBsAg QC is intended for monitoring system performance of the Access HBsAg and Access HBsAg Confirmatory assays. The Access HBsAg QC is for use on the DxI 9000 Access Immunoassay Analyzer.

For additional information, refer to the Access HBsAg and Access HBsAg Confirmatory reagent Instructions for Use.

SUMMARY AND EXPLANATION

Quality control (QC) materials simulate the characteristics of patient samples and are essential for monitoring the system performance of the Access HBsAg and Access HBsAg Confirmatory assays. In addition, they are an integral part of good laboratory practices. When performing assays with Access reagents for HBsAg, include quality control materials to validate the integrity of the assays. The assayed values should fall within the acceptable range if the test system is working properly.

TRACEABILITY

The analyte in the Access HBsAg QC is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of QC and are specific to the assay methodologies of the Access reagents. The values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

REAGENTS

CONTENTS

Access HBsAg QC
Ref. No. C39424: QC1-QC2: 4.0 mL/vial, 3 vials each level

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C. Do not use kit reagents beyond the expiration date.
- Vial is stable at 2 to 10°C for 90 days after initial use.
- Signs of possible deterioration are quality control values out of range.
- Refer to the QC value card for mean values and standard deviations (SD).

QC1:	Human serum negative for HBsAg, < 0.1% sodium azide, and 0.5% ProClin* 300.
QC2:	TRIS Buffer, human serum, HBs antigen, protein (bovine), < 0.1% sodium azide, and 0.5% ProClin* 300.
QC Value Card:	2

*ProClin is a trademark of LANXESS Corp.

Card 1 is for use with the Access HBsAg (REF C39422)

Card 2 is for use with the Access HBsAg Confirmatory (REF C39425)

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,[1] 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 regulations and guidelines.
- This product contains materials of human and animal origins and should be considered as potentially capable of transmitting infectious diseases.
- 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

ACCESS HBsAg QC1	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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
ACCESS HBsAg QC2	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.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

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

TESTING PROCEDURE(S)

PROCEDURE

Use the DxI 9000 Access Immunoassay analyzer to detect the presence of HBsAg in the Access HBsAg QC materials in the same manner as a sample. Include quality control materials in each 24-hour time period, or as required by individual laboratory procedures, because samples may be processed at any time in a “random access” format rather than a “batch” format. More frequent use of controls or the use of additional controls is left to the discretion of the operator, based upon good laboratory practices or the laboratory accreditation requirements and applicable laws. Refer to the appropriate system manuals and/or Help system for information on quality control theory, configuring controls, quality control sample test request entry, and reviewing quality control data.

To process the Access HBsAg QC in the Access HBsAg assay, 45 µL is required for each of the two levels in addition to the sample container and system dead volume (single determination).

To process the Access HBsAg QC in the Access HBsAg Confirmatory assay, 90 µL is required for the QC2 level (45 µL is required for the Control test and 45 µL is required for the Neutralization test) in addition to the sample container and system dead volume (single determination).

REPORTING RESULTS

EXPECTED RESULTS

For the Access HBsAg and Access HBsAg Confirmatory assays, the mean S/CO ratio and standard deviation (SD) are listed on the assay specific QC value card. The mean S/CO ratios should fall within the corresponding acceptable ranges. There are variations, such as technique, equipment, or reagents, which may cause results that are different from the listed values. Therefore, each laboratory should establish its own mean values and standard deviations (SD).

PROCEDURAL NOTES

LIMITATIONS

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

Initial release.

SYMBOLS KEY

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

REFERENCES

1. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication, 6th ed., June 2020.

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