Access
Immunoassay Systems

HBs Ag
REF A24291

Intended Use

The Access HBs Ag assay is a paramagnetic particle, chemiluminescent immunoassay for the qualitative detection of the surface antigen of the hepatitis B virus (HBs Ag) in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

The hepatitis B virus is responsible for hepatic lesions, such as acute hepatitis that can be fulminant and chronic hepatitis that can result in cirrhosis of the liver with a high risk of hepatocellular carcinoma.

Detection of the viral surface antigen (HBs Ag) in serum or plasma indicates an infection caused by the hepatitis B virus. It is the first marker to appear during the course of the disease and may be present two to three weeks in the blood before the clinical and biological symptoms of the disease. Presence of HBs Ag may last for a very short period (a few days) or a very long period (several years). If HBs Ag persists for more than six months, the hepatitis is classified as "chronic."

Because of the existence of numerous asymptomatic chronic carriers, screening for HBs Ag is required for each blood donation in order to prevent transmission of hepatitis by transfusion. In addition, prenatal screening of pregnant women has been recommended by health agencies so that the newborns from HBV carrier mothers may obtain prophylactic treatment.[1,2]

Principles of the Procedure

The Access HBs Ag assay is a one-step enzyme immunoassay ("sandwich") format. Neat sample is added to a reaction vessel with a specimen diluent solution. Paramagnetic particles coated with HBs Ag-specific monoclonal antibodies are added to the mixture followed by recombinant alkaline phosphatase conjugate coupled to another HBs Ag-specific monoclonal antibody. The HBs Ag-specific monoclonal antibodies were selected for their capacity to recognize the different HBs Ag subtypes and mutants.[3,4,5,6,7,8,9,10]

During the incubation, HBs Ag present in the sample is captured by both the solid phase and the 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 (Lumi-Phos* 530) is added and the relative light unit (RLU) generated by the enzyme reaction is measured with a luminometer. The photon production is a function of the amount of enzyme conjugate present at the end of the reaction. The quantity of measured light for a sample indicates the presence or absence of HBs Ag by comparison with a cut-off value determined during the assay calibration on the instrument. If the photon production is equal to or higher than the cut-off value, the sample is considered as reactive for HBs Ag. A reactive sample must be processed as described in the Results section.

Product Information

Access HBs Ag Reagent Pack

Cat. No. A24291: 100 tests, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- 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.
- Stable at 2 to 10°C for 56 days after initial use.

- 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 (i.e., broken elastomer), discard the pack.

R1a:	Dynabeads** paramagnetic particles coated with streptavidin and coupled to biotinylated monoclonal (mouse) HBs Ag specific antibodies in a TRIS buffer with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.25% ProClin*** 300.
R1b:	TRIS buffer with surfactant, protein (mouse, bovine), < 0.1% sodium azide, and 0.25% ProClin 300.
R1c:	Alkaline phosphatase (recombinant) conjugated monoclonal (mouse) HBs Ag specific antibody in phosphate buffer with surfactant, BSA, < 0.1% sodium azide, and 0.25% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient 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, 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.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin, EDTA, ACD and sodium citrate) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[12]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Centrifuge the samples.
   - Keep tubes stoppered at all times.
   - Store samples tightly stoppered at room temperature (20 to 25°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
3. If the assay will not be completed within 4 days, or for shipment of samples, freeze at -20°C or colder.
4. Use the following guidelines when preparing specimens:
   - In general, allow 1 hour for serum samples to clot completely.
   - All samples stored longer than 8 hours should be centrifuged at 3000 g for 15 minutes prior to testing.
   - Follow blood collection tube manufacturer's recommendations or validated laboratory procedures for centrifugation.
5. Ensure residual fibrin and cellular matter have been removed prior to analysis. Turbid serum or plasma samples containing particulate matter should be transferred from the original tube

and re-centrifuged prior to assay. A specimen (original tube) that contains a separating device (gel barrier) is never to be re-centrifuged.

6. If a sample is not to be assayed within 2 hours after centrifugation, transfer the cell-free sample to a storage tube (at least 550 µL sample volume is required plus sample container and system dead volumes to allow sufficient volume for confirmatory testing algorithm if needed). Tightly stopper the tube immediately. Refer to the appropriate system manuals and/or Help system for a specific description of each instrument's dead volume requirements.
7. Samples must be mixed thoroughly after thawing. Remove the suspended fibrin particles or aggregates by centrifugation at 3000 g for 15 minutes. Transfer the sample into a sample cup for testing.
8. Use caution in handling patient specimens to prevent cross-contamination.
9. Thaw samples no more than 5 times.
10. No qualitative differences in the results were found after 25 non-reactive samples and 25 reactive samples were heated at 56°C for 30 minutes.

Materials Provided

R1 Access HBs Ag Reagent Packs

Materials Required But Not Provided

1. Access HBs Ag Calibrators
   Negative and positive for HBs Ag
   Cat. No. A24292
2. Quality Control (QC) materials: Access HBs Ag QC or other commercially available quality control material.
   Cat. No. A24294
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

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. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use one hundred ten (110) 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.
4. The system default unit of measure for sample results is Signal/Cutoff (S/CO) ratio.
5. Time to first result is approximately 55 minutes.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

Results of the Access HBs Ag Calibrators are automatically checked by the Access system against pre-defined RLU limits. If the RLUs meet pre-defined specifications, the system calculates the calibration cut-off value. An active calibration curve is required for all tests. For the Access HBs Ag assay, calibration is required every 56 days for each new reagent pack lot.

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.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[13] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Include Access HBs Ag QC or other commercially available quality control materials that cover at least two levels of analyte. Follow manufacturer's instructions for reconstitution and storage. 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 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.

Results

The presence or absence of HBs antigen is determined by comparing the RLU signal of each patient specimen to the calibration cut-off value. Signal to cut-off ratio (S/CO) = Sample RLU / Calibration cut-off RLU. Results are reported to be "reactive" or "non-reactive" as a function of their ratio with the cut-off value (signal greater than or signal equal to or less than the cut-off value, respectively). However, results ~ 10% lower than the "cut-off value" should be prudently interpreted and retested in duplicate. This gray zone (from 0.9 to less than 1.0) must be stored by the user (refer to the appropriate system manuals and/or Help system for complete instructions on gray zone for a qualitative assay). In this way a distinctive mark automatically will be reported, permitting rapid identification of a result situated in the gray zone. Patient 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.

Any sample found "reactive" in the first test should be retested in duplicate after transfer of the serum or plasma to a tube (the primary tube should not be re-centrifuged). Centrifuge the tube containing the sample for 15 minutes at 3000 g and then transfer the sample supernatant to a sample cup.

- **All samples that are initially reactive or with results in the gray zone should be retested in duplicate using the Access HBs Ag assay. If 2 of 3 results are < 1.0 S/CO, the sample must be considered non-reactive (negative) for HBs Ag. If 2 of 3 results are ≥ 1.0 S/CO, the samples must be considered repeatedly reactive.**
- **Repeatedly reactive samples should be confirmed using the Access HBs Ag Confirmatory assay. Samples that are confirmed using the neutralization reagent (containing human HBs Ag-specific IgG) must be considered positive for HBs Ag.**

Limitations of the Procedure

1. The Access HBs Ag assay is strictly limited to the detection of the surface antigen of the hepatitis B virus in human serum and plasma (heparin, EDTA, ACD and sodium citrate). Results obtained with the Access HBs Ag assay must be consistent with the symptoms and the clinical history. A reactive result obtained with the Access HBs Ag assay should be confirmed by the Access HBs Ag Confirmatory assay before reporting an infection.

   In addition, several authors have reported in the literature cases of viral hepatitis B (acute or chronic) wherein viral DNA is detectable in the absence of the surface antigen (HBs Ag negative patients). These abnormal profiles, though rare, are the consequence of possible genetic mutations, either at the S and pre-S gene level (preventing recognition of the Ag by some immunological reagents) or, usually, at the X and pol gene level, inducing weak viral replication. Testing additional markers (HBs Ag-specific antibody or, if possible, amplified viral DNA) is recommended for the final diagnosis of the infection, in those very particular cases. [14,15]

2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[16,17]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access HBs Ag 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.
4. The Access HBs Ag assay does not demonstrate any "hook" effect up to 5 mg/mL.

Specific Performance Characteristics

Intra-Assay Precision

The intra-assay precision was determined by testing five different samples 30 times in the same run during one day. The results of this study are shown below:

Table 1: Intra-assay Precision of the Access HBs Ag Assay

Sample	Mean (S/CO)	SD	%CV
1 (Neg)	0.16	0.01	N/A†
2	0.97	0.02	1.8
3	2.73	0.07	2.4
4	8.31	0.20	2.4
5	27.07	0.41	1.5

Inter-Assay Precision

The inter-assay precision was determined by testing five different samples in duplicate for 20 days, 2 runs per day. The results of this study are shown below:

Table 2: Inter-assay Precision of the Access HBs Ag Assay

Sample	Mean (S/CO)	SD	%CV
1 (Neg)	0.17	0.02	N/A†
2	1.01	0.07	7.5
3	2.82	0.16	5.9
4	8.54	0.43	5.4
5	27.36	1.16	4.3

The Access HBs Ag assay exhibits total imprecision of ≤ 10% with reactive samples.

† Due to low S/CO value, %CV is not applicable.

Specificity

Specificity was determined by testing samples that were found negative in a reference assay and tested in the Access HBs Ag assay. All samples that were found to be repeatedly reactive in the Access assay were run in the corresponding Access HBs Ag Confirmatory assay.

In one study, a total of 5020 samples were tested. These samples were comprised of volunteer blood donors from 2 sites. The specificity on the volunteer blood donor population was 99.96%. The results of this study are shown below.

Table 3: Specificity of the Access HBs Ag Assay on Samples from Volunteer Blood Donors

Sample Category	Number of Samples Tested	Number of Repeat Reactive Samples	Specificity (%)
Volunteer Blood Donors	5020	2 (0.04%)	99.96 (95% CI: 99.86–100)

In another study, 565 samples from a hospital population were tested. These samples were from subjects who were tested for HBs Ag as part of their routine medical care (i.e.: samples submitted to the hospital laboratory for diagnostic testing). Samples in this study included subjects with signs and symptoms of hepatitis and subjects at risk of HBV infection or with suspected exposure to HBV. The specificity on the hospital population was 99.47%. The results of this study are shown below.

Table 4: Specificity of the Access HBs Ag Assay on Samples from a Hospital Population

Sample Category	Number of Samples Tested	Number of Repeat Reactive Samples	Specificity (%)
Hospital Population	565	3 (0.5%)	99.47 (95% CI: 98.45–99.82)

Analytical Sensitivity

The analytical sensitivity of the Access HBs Ag assay was evaluated with an internal panel titrated against the French Blood Transfusion Society (SFTS) 2005 panel and was determined to be ≤ 0.1 ng/mL.

The analytical sensitivity was also evaluated with the WHO NIBSC 80/549 (first) and 00/588 (second) international standards, and the PEI subtype (ad and ay) panel. The analytical sensitivity with each of these standards from individual studies is listed in the table below.

Table 5: Analytical Sensitivity of the Access HBs Ag Assay

Standard	Analytical Sensitivity
WHO NIBSC 80/549	0.092 IU/mL (95% CI: 0.083–0.103)
WHO NIBSC 00/588	0.056 IU/mL (95% CI: 0.054–0.059)
PEI ad	0.020 PEI Units/mL (95% CI: 0.011–0.030)
PEI ay	0.024 PEI Units/mL (95% CI: 0.013–0.038)

Sensitivity

Sensitivity was determined by testing samples that were found positive in a reference assay and tested in the Access HBs Ag assay. All samples found to be repeatedly reactive were run in the corresponding Access HBs Ag Confirmatory assay.

A total of 410 samples including subjects with acute and chronic hepatitis were tested. The sensitivity on this population was 100% (95% CI: 99.27–100). The results of the study are shown below.

Table 6: Sensitivity of the Access HBs Ag Assay

Sample Category	Number of Samples Tested	Number of Reactive Samples	Sensitivity (%)
HBs Ag Positive	410	410	100% (95% CI: 99.27–100)

Thirty well-documented commercial HBV seroconversion panels were tested with the Access HBs Ag assay and a reference assay. The Access HBs Ag assay exhibited higher sensitivity with 10 panels, equivalent sensitivity with 18 panels, and lower sensitivity with 2 panels.

To evaluate subtype and genotype recognition, a SFTS subtype panel and a Teragenix genotype panel were tested. The Access HBs Ag assay detected all subtypes (adw2, adw4, adr, ayw1, ayw2, ayw3, ayw4 and ayr) and all genotypes (A-1, B-6, C-2, D-27, E-23, F-18 and G-50).

In addition, a panel of 15 recombinant proteins representing major mutations on amino acid sequences of the HBs Ag antigen were tested. All recombinant panel samples were detected with the Access HBs Ag assay.

Cross Reactivity

A study of 293 samples from potentially cross-reacting medical conditions including, HCV, HSV IgG and IgM, Rubella IgG and IgM, Toxo IgG and IgM, HTLV 1-2, Mumps IgG, CMV IgG, EBV IgG and IgM, Measles IgG and IgM, Rheumatoid Factor (RF), ANA, HAV IgG and IgM, HIV, renal failure patients, dialysis patients, Myeloma, Influenza, HAMA, and alcoholic cirrhosis was performed. None of the samples that were tested showed cross reactivity with the exception of RF (1 out of 17), and ANA (1 out of 36), which were repeat reactive but did not confirm with the Access HBs Ag Confirmatory assay.

Interference samples containing up to 300 mg/L bilirubin (100 mg/L free and 200 mg/L conjugated), lipemic samples containing 30 g/L triolein (triglyceride), 90 g/L albumin, and hemolyzed samples containing up to 5 g/L hemoglobin do not affect the result.

Access
Immunoassay Systems

HBs Ag CALIBRATORS
REF A24292

Intended Use

The Access HBs Ag Calibrators are intended for use with the Access HBs Ag and Access HBs Ag Confirmatory assays for the qualitative detection and confirmation, respectively, of the presence of the hepatitis B virus surface antigen (HBs Ag) in human serum or plasma using the Access Immunoassay Systems.

Summary and Explanation

The Access HBs Ag Calibrators are used to establish calibration (determine the cut-off value) for the Access HBs Ag and Access HBs Ag 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 measurand (analyte) in the Access HBs Ag Calibrators 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 calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access HBs Ag Calibrators

Cat. No. A24292: C0 and C1, 2.7 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 unopened 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 calibration card for exact concentrations.

C0:	Negative calibrator: Buffered BSA matrix, < 0.1% NaN_3, 0.25% ProClin*** 300.
C1:	Positive calibrator: Buffered BSA matrix, HBs antigen, < 0.1% NaN_3, 0.25% ProClin 300.
Calibration Cards:	2

Note:

- Card 1 is for use with the product code A24291.
- Card 2 is for use with the product code A24295.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient 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, 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.

- The antigen used in the preparation of the reagent is derived from human serum/plasma. Handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, 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.[18]
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

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.

Calibration Details

The Access HBs Ag Calibrators are provided as: negative and positive. The Access HBs Ag and Access HBs Ag Confirmatory assays each require a calibration (determination of the cut-off value) in order to have an active "calibration." Each calibration requires 220 µL of each of the two calibrators (each in duplicate) in addition to the sample container and system dead volume. One drop is equal to approximately 40 µL.

Refer to the appropriate system manuals and/or Help systems for the minimum sample volume required.

Limitations of the Procedure

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

Access
Immunoassay Systems

HBs Ag QC
REF A24294

Intended Use

The Access HBs Ag QC is intended for monitoring system performance of the Access HBs Ag and Access HBs Ag Confirmatory assays.

Summary and Explanation

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of the Access HBs Ag and Access HBs Ag Confirmatory immunoassays. In addition, they are an integral part of good laboratory practices.[13,19,20,21,22,23] When performing assays with Access reagents for Access HBs Ag and Access HBs Ag Confirmatory, 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 measurand (analyte) in the Access HBs Ag 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. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access HBs Ag QC

Cat. No. A24294: QC1 and QC2: 4.0 mL/vial, 3 vials each level

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C or store frozen at -20°C or colder.
- Stable until the expiration date stated on the label when stored unopened at 2 to 10°C or at -20°C. Do not use kit reagents beyond the expiration date.
- Vial is stable at 2 to 10°C for 56 days after initial use.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Signs of possible deterioration are quality control values out of range.

QC1:	Human defibrinated plasma, HBs Ag negative, < 0.1% NaN_3, 0.25% ProClin*** 300.
QC2:	Human defibrinated plasma, HBs Ag positive, < 0.1% NaN_3, 0.25% ProClin 300.
QC Value Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient 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, 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.
- The antigen used in the preparation of the reagent is derived from human serum/plasma. Handle these products as potentially infectious according to universal precautions and good

clinical laboratory practices, 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.[18]

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

The Access HBs Ag QC should be treated in the same way as patient specimens and run in accordance with the instructions accompanying the instrument and/or method being used. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[13] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

Note: For the Access Immunoassay Systems, refer to the appropriate system manuals and/or Help system for information on quality control theory, configuring quality controls, quality control sample test request entry, and reviewing quality control data.

To process the Access HBs Ag QC in the Access HBs Ag assay, 110 µL of sample is required for each of the two levels in addition to the sample container and system dead volume (single determination). One drop is equal to approximately 40 µL.

To process the Access HBs Ag QC in the Access HBs Ag Confirmatory assay, 110 µL is required for the Control test and 110 µL is required for the Neutralization test for each of the two levels in addition to the sample container and system dead volume (single determination). One drop is equal to approximately 40 µL.

Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

Limitations of the Procedure

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

Expected Values

For the Access HBs Ag assay, the expected mean S/CO ratio ($\bar{x}$) and SD (σ) for the Access HBs Ag QC levels (QC1 and QC2) are provided on the QC value card. Individual S/CO ratios should fall within the corresponding acceptable ranges. However, each laboratory should assign its own S/CO mean and acceptable ranges after sufficient data have been collected.[13,23]

For the Access HBs Ag assay, quality control data should be:

QC1: Non-Reactive

QC2: Reactive

For the Access HBs Ag Confirmatory assay, quality control data should be:

	Control	Neutralization
QC1	Non-Reactive	Not Confirmed
QC2	Reactive	Confirmed

Access
Immunoassay Systems

HBs Ag CONFIRMATORY
REF A24295

Intended Use

The Access HBs Ag Confirmatory assay is a paramagnetic particle, chemiluminescent immunoassay for the confirmation of the presence of hepatitis B surface antigen (HBs Ag) in human serum and plasma specimens that have been found to be repeatedly reactive in the Access HBs Ag assay. The Access HBs Ag Confirmatory assay is intended for use with the Access Immunoassay Systems.

Principles of the Procedure

The Access HBs Ag Confirmatory assay uses the principle of neutralization by an excess of HBs Ag-specific antibodies (neutralization reagent) to confirm the presence of HBs Ag in serum or plasma that is found to be repeatedly reactive in the Access HBs Ag assay. The confirmatory assay reports % inhibition (suppression) of a control assay (HBs Ct) with a neutralizing reagent (HBs Bk).

Neat sample is added to two separate reaction vessels, one containing the neutralization reagent (human HBs Ag-specific IgG), and the other containing the specimen diluent solution. Paramagnetic particles coated with HBs Ag-specific monoclonal antibodies are added to the mixture followed by recombinant alkaline phosphatase conjugate coupled to HBs Ag-specific monoclonal antibody. The HBs Ag-specific monoclonal antibodies were selected for their capacity to recognize the different HBs Ag subtypes and mutants.[3,4,5,6,7,8,9,10]

If HBs Ag is present in the sample, the HBs Ag-specific antibodies of the neutralization reagent bind to the antigenic determinants that can no longer bind to the antibodies of 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 (Lumi-Phos* 530) is added and light generated by the enzyme reaction is measured with a luminometer. The photon production is a function of the amount of enzyme conjugate present at the end of the reaction. Any reduction of the generated light is recorded by comparison with the same sample in which the neutralization reagent is replaced by the specimen diluent solution.

Both neutralization and specimen diluent control tests are automatically run by the Access Immunoassay Systems.

Product Information

Access HBs Ag Confirmatory Reagent Pack

Cat. No. A24295: 100 tests (sufficient for 50 patient samples), 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- 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.
- Stable at 2 to 10°C for 56 days after initial use.
- 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 (i.e., broken elastomer), discard the pack.

R1a:	Dynabeads** paramagnetic particles coated with streptavidin and coupled to biotinylated monoclonal (mouse) HBs Ag specific antibodies in a TRIS buffer with BSA, < 0.1% sodium azide, and 0.25% ProClin*** 300.
R1b:	Human gamma globulins specific for HBs Ag in TRIS buffer with surfactant, protein (mouse, bovine), < 0.1% sodium azide, 0.25% ProClin 300.
R1c:	TRIS buffer with surfactant, protein (mouse, bovine), < 0.1% sodium azide, and 0.25% ProClin 300.
R1d:	Alkaline phosphatase (recombinant) conjugated monoclonal (mouse) HBs Ag specific antibody in phosphate buffer with surfactant, BSA, < 0.1% sodium azide, and 0.25% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient 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, 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.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin, EDTA, ACD and sodium citrate) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[12]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Centrifuge the samples.
   - Keep tubes stoppered at all times.
   - Store samples tightly stoppered at room temperature (20 to 25°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
3. If the assay will not be completed within 4 days, or for shipment of samples, freeze at -20°C or colder.
4. Use the following guidelines when preparing specimens:
   - In general, allow 1 hour for serum samples to clot completely.
   - All samples stored longer than 8 hours should be centrifuged at 3000 g for 15 minutes prior to testing.
   - Follow blood collection tube manufacturer's recommendations or validated laboratory procedures for centrifugation.
5. Ensure residual fibrin and cellular matter have been removed prior to analysis. Turbid serum or plasma samples containing particulate matter should be transferred from the original tube and re-centrifuged prior to assay. A specimen (original tube) that contains a separating device (gel barrier) is never to be re-centrifuged.

6. If a sample is not to be assayed within 2 hours after centrifugation, transfer the cell-free sample to a storage tube (at least 550 µL sample volume is required plus sample container and system dead volumes to allow sufficient volume for confirmatory testing algorithm if needed). Tightly stopper the tube immediately. Refer to the appropriate system manuals and/or Help system for a specific description of each instrument's dead volume requirements.
7. Samples must be mixed thoroughly after thawing. Remove the suspended fibrin particles or aggregates by centrifugation at 3000 g for 15 minutes. Transfer the sample into a sample cup for testing.
8. Use caution in handling patient specimens to prevent cross-contamination.
9. Thaw samples no more than 5 times.
10. No qualitative differences in the results were found after 25 non-reactive and 25 reactive samples were heated at 56°C for 30 minutes.

Materials Provided

R1 Access HBs Ag Confirmatory Reagent Packs

Materials Required But Not Provided

1. Access HBs Ag Calibrators
   Negative and positive for HBs Ag
   Cat. No. A24292
2. Quality Control (QC) materials: Access HBs Ag QC or other commercially available quality control material.
   Cat. No. A24294
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

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. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. **Caution:** To ensure results are calculated correctly, always assign a unique Sample ID to patient samples that have been diluted offline prior to analysis.
4. The assay uses the Ag neutralization principle. Samples found to be reproducibly reactive with the Access HBs Ag assay are incubated with and without neutralizing reagent (human HBs Ag-specific IgG). Samples containing neutralized HBs Ag produce lower RLU than non-neutralized samples. The % inhibition is used to determine if positive samples are confirmed.
5. Calibrate the confirmatory control assay using the Access HBs Ag Calibrators and the confirmatory calibration card.
   Note: The calibrator kit contains two calibration cards; one for Access HBs Ag and one for Access HBs Ag Confirmatory.
6. The Access HBs Ag QC can be used for the Access HBs Ag and Access HBs Ag Confirmatory assays.
7. Once an active curve is established for the HBsCt assay, program the instrument for both the HBsBk assay and the HBsCt assay. The HBsBk assay is measured by percent inhibition and the HBsCt assay is measured by S/CO.

8. The HBsBk and the HBsCt assays each require 110 µL of sample 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.
9. Time to first result is 55 minutes.
10. The S/CO ratio is determined by the active calibration and the % of inhibition is calculated by: [1 - (HBsBk RLU / HBsCt RLU)] x 100.

S/CO ratio	% Inhibition	Interpretation
< 1.0	Not applicable	–
≥ 1.0	≥ 40	Confirmed Reactive
≥ 1.0	< 40	To be diluted or Not Confirmed

11. If the ratio is greater or equal to 1.0 and the % of inhibition is less than 40%, dilute the sample with wash buffer and rerun on the HBsBk and the HBsCt assays. Do not enter the dilution factor in the Dilution Factor Field since this is a qualitative assay.
12. Repeatedly reactive samples with S/CO ratios greater than or equal to 150 in the Access HBs Ag can be directly diluted and tested with the Access HBs Ag Confirmatory assay. However, should the result indicate that a specimen may have been over-diluted (i.e. signal to cut-off < 1.0 for the Specimen Diluent Control), a more concentration sample should be prepared and tested.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

Results of the Access HBs Ag Calibrators are automatically checked by the Access system against pre-defined RLU limits. If the RLUs meet pre-defined specifications, the system calculates the calibration cut-off value. An active calibration cut-off value is required for all tests. For the Access HBs Ag Confirmatory assay, calibration is required every 56 days or for each new reagent pack lot. 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.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[13] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Include commercially available quality control materials that cover at least two levels of analyte. Follow manufacturer's instructions for reconstitution and storage. 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 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.

Results

Patient test results are determined automatically by the system software. A sample is confirmed positive if calculations performed by the Access system show a signal/cut-off ratio greater than or equal to 1.0 and a % inhibition greater than or equal to 40%. The cut-off value is determined by active calibration and the percent of inhibition by: [1.0 - (Neutralization Reagent Test Value/Specimen Diluent Control Test Value)] x 100. Samples with a S/CO ratio of ≥ 1.0 and a % inhibition of < 40% may need to be diluted up to 1/10,000 as indicated below.

Neat Sample

Specimen Diluent Control signal/cut-off	% Inhibition or Suppression (% Supp)	Interpretation
< 1.0	Not applicable	Non-Reactive for HBs Ag
≥ 1.0	≥ 40	Confirmed positive
≥ 1.0	< 40	To be diluted 1/100

Diluted Sample (e.g. 1/100)

Specimen Diluent Control signal/cut-off	% Inhibition or Suppression (% Supp)	Interpretation
< 1.0	Not applicable	Non confirmed reactive
≥ 1.0	≥ 40	Confirmed positive
≥ 1.0	< 40	To be diluted 1/1000

Remarks

- Rarely, a 1/10,000 dilution is necessary to confirm whether the sample is true positive.
- All sample dilutions are done with the Access Wash Buffer II.
- **Caution**: To ensure results are calculated correctly, always assign a unique Sample ID to patient samples that have been diluted offline prior to analysis.

Limitations of the Procedure

The Access HBs Ag Confirmatory assay is strictly limited to the confirmation of the presence of the surface antigen of the hepatitis B virus in human serum or plasma. 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.

Specific Performance Characteristics

Intra-Assay Precision

The intra-assay precision was determined by testing four different samples 30 times in the same run during one day. The results of this study are shown below:

Table 1: Intra-assay Precision of the Access HBs Ag Confirmatory Assay

Sample	Mean (S/CO)	% Inhibition	%CV (Inhibition)
1	1.0	74.6	0.4
2	2.9	86.0	0.3
3	8.4	82.3	0.5
4	27.1	86.7	0.4

Inter-Assay Precision

The inter-assay precision was determined by testing four different samples in duplicate for 20 days, 2 runs per day. The results of this study are shown below:

Table 2: Inter-assay Precision of the Access HBs Ag Confirmatory Assay

Sample	Mean (S/CO)	% Inhibition	%CV (Inhibition)
1	1.0	74.0	1.7
2	2.8	85.8	0.5
3	8.5	82.7	0.6
4	27.4	86.7	0.4

The Access HBs Ag Confirmatory assay exhibits total imprecision of ≤ 10% with reactive samples.

Specificity

Specificity was determined by testing repeatedly reactive samples found to be negative for HBs Ag in a reference assay. None of these samples were confirmed by the Access HBs Ag Confirmatory assay.

Analytical Sensitivity

All sample concentrations from the SFTS 2005 panel, the WHO NIBSC 80/549 (first) and 00/588 (second) international standards, and the PEI subtype (ad and ay) panel were confirmed as positive with the Access HBs Ag Confirmatory assay. The lowest concentration tested with each standard is listed in the table below:

Table 3: Analytical Sensitivity of the Access HBs Ag Confirmatory Assay

Standard	Lowest Concentration Tested
SFTS 2005	0.11 ng/mL
WHO NIBSC 80/549	0.125 IU/mL
WHO NIBSC 00/588	0.062 IU/mL
PEI ad	0.031 PEI Units/mL
PEI ay	0.031 PEI Units/mL

Sensitivity

Sensitivity was determined by testing samples in the Access HBs Ag assay and in another commercially available HBs Ag assay. All repeatedly reactive samples were run in the corresponding HBs Ag Confirmatory assay.

A total of 413 samples including subjects with acute and chronic hepatitis were tested. Out of 413 repeat reactive samples, 413 were confirmed with the Access HBs Ag Confirmatory assay.

Twenty well-documented commercial HBV seroconversion panels were tested. All reactive samples were confirmed with the Access HBs Ag Confirmatory assay.

To evaluate subtype and genotype recognition, a SFTS subtype panel and a Teragenix genotype panel were tested. All subtypes (adw2, adw4, adr, ayw1, ayw2, ayw3, ayw4 and ayr) and all genotypes (A-1, B-6, C-2, D-27, E-23, F-18 and G-50) were confirmed with the Access HBs Ag Confirmatory assay.

In addition, a panel of 15 recombinant proteins representing major mutations on amino acid sequences of the HBs antigen were tested. All recombinant panel samples were confirmed with the Access HBs Ag Confirmatory assay.

Cross Reactivity

Cross reactivity was determined by spiking HBs Ag into 120 samples originating from potentially cross-reacting medical conditions including HAV IgG and IgM, HCV, HIV, renal failure patients, dialysis patients, Myeloma, Influenza, HAMA, alcoholic cirrhosis, Rheumatoid Factor (RF), and ANA. All spiked samples were confirmed with the Access HBs Ag Confirmatory assay.

Interference samples containing up to 300 mg/L bilirubin (100 mg/L free and 200 mg/L conjugated), lipemic samples containing 30 g/L triolein (triglyceride), 90 g/L albumin and hemolyzed samples containing up to 5 g/L hemoglobin do not affect the result.

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