Laboratory Procedure Manual

Analyte: Hepatitis B Surface Antigen (HBsAg)

Matrix: Serum

Method: *HBsAg* VITROS Immunodiagnostic Products

Method No.:

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As performed by: *Assay Development and Diagnostic Reference Laboratory*
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Important Information for Users

The National Center for HIV/AIDS, Hepatitis, STD and TB Prevention (NCHHSTP) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HEPBD_G	LBDHBG	Hepatitis B surface antigen

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The VITROS HBsAg test is performed using the VITROS HBsAg Reagent Pack and VITROS Immunodiagnostic Products HBsAg Calibrator on the VITROS ECi/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System. An immunometric immunoassay technique is used, which involves the simultaneous reaction of HBsAg in the sample with mouse monoclonal anti-HBs antibody coated onto the wells and a horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBs antibody in the conjugate. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indicative of the level of HBsAg present in the sample.

Viral hepatitis is a major public health problem of global importance with an estimated 300 million persistent carriers of hepatitis B virus (HBV) worldwide. Infection with HBV results in a wide spectrum of acute and chronic liver diseases that may lead to cirrhosis and hepatocellular carcinoma.

Viral hepatitis is a disease of the liver that is caused by a number of well-characterized viruses including HBV. Transmission of HBV occurs by percutaneous exposure to blood products and contaminated instruments, sexual contact and perinatally from HBV-infected mothers to their unborn child.

HBV infection produces an array of unique antigens and antibody responses that, in general, follow distinct serological patterns. Hepatitis B surface antigen (HBsAg), derived from the viral envelope, is the first antigen to appear following infection and can be detected serologically as an aid in the laboratory diagnosis of acute HBV infection.

Detection of HBsAg by sensitive enzyme immunoassays was described by Engvall and Perlmann, Engvall, Jonsson and Perlmann, and VanWeemen and Schuurs in 1971. Subsequently, solid-phase sandwich enzyme immunoassays for the detection of HBsAg were described by Wisdom, Wolters et al. and Wei et al. Production, characterization and application of monoclonal antibodies for the detection of HBsAg have also been described.

2. SAFETY PRECAUTIONS:

Test kits for HBsAg contain components derived from human serum or plasma. Although various treatments in the manufacturing process are sufficient to inactivate most blood-borne pathogens, there is no assurance that these reagents are entirely noninfectious. Therefore, treat components of test kits as though they are capable of transmitting disease.

Consider all serum specimens for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of infectious contamination hazards. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished. Biosafety Level 2 containment and practice as described in CDC/NIH publication #88-8395 are recommended for handling test specimens and kit reagents.

The VITROS HBsAg conjugate reagent and assay reagent pack contain Kathon. May cause sensitization by skin contact. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Avoid contact with skin. Wear suitable gloves

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. The run information can be uploaded into the computerized database after the run information is exported by the software to the computerized database. This database was custom-designed for the management of CDC Assay Development and Diagnostic Reference Laboratory (ADDRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. Test values are compared with a cutoff value. Results are expressed as "positive" or "negative" for HBsAg. Other information in the database may typically include the ADDRL identification number, the specimen number, the date collected, the date tested, and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer.

b. Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with the other NHANES IV data.

c. Files stored on the LAN are automatically backed up nightly to tape by CDC Data Center staff.

d. Documentation for data system maintenance is contained in hard copies of data records for 2 years.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

No special patient preparation is necessary.
Specimens Recommended: Serum

Do not use turbid specimens. Turbidity in specimens may affect test results.

Collect specimens using standard procedures

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.

Thoroughly mix samples by inversion and bring to 15–30°C (59–86°F) before use.

The VITROS HBsAg test uses 80 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.

The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. For detailed information refer to the operating instructions for your system.

The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing blood specimens:

- Store samples at 22°C (72°F) for no longer than 8 hours.
- If the test will not be completed within 8 hours, refrigerate the sample at 2–8°C (36–46°F).
- If the test will not be completed within 48 hours, or for shipment of samples, freeze at or below –20°C (-4°F).

Samples are not to be repeatedly frozen and thawed because this can cause analyte deterioration. Samples are to be thawed only once.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Required Materials not Provided

VITROS Immunodiagnostic Products Signal Reagent
VITROS Immunodiagnostic Products Universal Wash Reagent
Quality control materials such as VITROS Immunodiagnostic Products HBsAg Controls
VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

b. Materials Provided

- VITROS Immunodiagnostic Products HBsAg Reagent Pack
- VITROS Immunodiagnostic Products HBsAg Calibrator

c. Reagent Preparation

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (mouse monoclonal anti-HBs (directed to the “a” region determinant), coated at 1 µg/well)
- 6.2 mL conjugate reagent (HRP- mouse monoclonal anti-HBs, 0.9 µg/mL) in buffer with bovine serum albumin, goat serum, and antimicrobial agent (Kathon 1% w/v)
- 8.4 mL assay reagent with human serum, newborn calf serum, mouse serum and antimicrobial agent (Kathon 1% w/v)

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading onto the system.
- Handle the reagent pack with care. Avoid the following:
  - allowing condensation to form on the pack
  - causing reagents to foam
  - agitation of the pack

Reagent Pack Storage and Preparation

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8°C (36–46°F)	expiration date
Opened	On system	System turned on	8 weeks
Opened	Refrigerated	2–8°C (36–46°F)	8 weeks

- The VITROS HBsAg Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.
- Exposure of Reagent Pack and Calibrator to temperatures >30°C (86°F) for extended periods of time may affect test performance.

d. Standards Preparation

This method does not involve the use of conventional calibrators or standards. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and Integrated Systems.

e. Preparation of Quality Control Material

(1) Kit positive and negative controls are prepared and quality controlled by the manufacturer.

(2) In-house controls are prepared according to ADDRL specifications.

f. Calibrators

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System for the qualitative *in vitro* detection of hepatitis B surface antigen (HBsAg) in human serum and plasma using VITROS HBsAg Reagent Packs. The VITROS HBsAg Calibrator has been validated for use only on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System with the VITROS Immunodiagnostic Products HBsAg Reagent Pack

Calibrator Contents

- VITROS HBsAg Calibrator (human HBsAg ad subtype, inactivated, 2mL; 0.70±0.30 PEI Units* /mL) in buffer with bovine serum albumin and antimicrobial agent
- Lot calibration card
- Protocol card
- 8 calibrator bar code labels

* Paul-Ehrlich-Institute HBsAg reference serum

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30°C (59–86°F) before use. Each pack contains sufficient for a minimum of 6 determinations of each calibrator.
- Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8°C (36–46°F) as soon as possible after use, or load only sufficient for a single determination.

Calibrator Storage and Preparation

Calibrator	**Storage Condition**		**Stability**
Unopened	Refrigerate	2–8°C (36-6°F)	expiration date
Opened	Refrigerate	2–8°C 36-46°F)	12 weeks
Opened	Frozen	-20°C (-4°F)	12 weeks

- The VITROS HBsAg Calibrator is supplied ready for use.
- The VITROS HBsAg Calibrator is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Opened calibrators may be stored frozen (with no more than 1 freeze-thaw cycle).

- The VITROS HBsAg test uses 80 µL of calibrator for each determination. The VITROS HBsAg Calibrators may be used directly on the VITROS Immunodiagnostic and VITROS Integrated Systems. Alternatively, transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.
- The VITROS HBsAg Calibrator is automatically processed in duplicate.

g. Instrument

VITROS ECi/ECiQ and VITROS 3600 Immunodiagnostic System

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Procedure

- Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration is established for each new reagent lot by performing multiple tests. This is the process by which a lot-specific parameter [a] which links the signal at the cutoff (cutoff value) to the calibrator signal is determined.
  Cutoff value = (a x Signal of Cal 1)
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process the calibrator in the same manner as samples. Load sufficient for the automatic duplicate determination. Calibration need not be programmed if bar code labels are used; Calibration will be initiated automatically.
- When the calibrator is processed the validity of the calibration is assessed against quality parameters which compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cutoff value is calculated and stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a quality parameter. Failure to meet the defined quality parameter range will be coded in the calibration report. For actions to be taken following a failed calibration, refer to the operating instructions for your system.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

b. When to Calibrate

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

c. Traceability of Calibration
The calibration of the VITROS HBsAg test is traceable to an in-house reference calibrator which has been value-assigned to optimize the clinical sensitivity and specificity performance.

d. Calibration Model
Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and VITROS Integrated Systems.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) The VITROS HBsAg Reagent Pack is used for 100 tests. Kit components cannot be interchanged within a manufacturer's lot or between lots.
(2) Unopened reagent pack is stored refrigerated at 2-8°C; do not freeze.
(3) Reagent pack is loaded on the instrument directly from refrigerated storage to minimize condensation.
(4) Prepare a runsheet, listing controls and specimens in the order presented in the e-file.
(5) Perform daily maintenance of the VITROS instruments according to user manual, verifying the validity of the calibrators and if needed update. Run negative and positive controls.

b. Sample Preparation

(1) Bring serum specimens and controls from the refrigerator to the bench, mix each vial by inversion and allow 20-30 minutes to reach ambient temperature (15-30°C).

Spin down the specimens at 5000 RPM speed for 5 minutes using a swing-bucket centrifuge (Eppendorf Centrifuge 5804/Rotor A-4-44, or similar).

(2) Identify the reaction tray wells for each specimen or control.

c. Instrument Setup

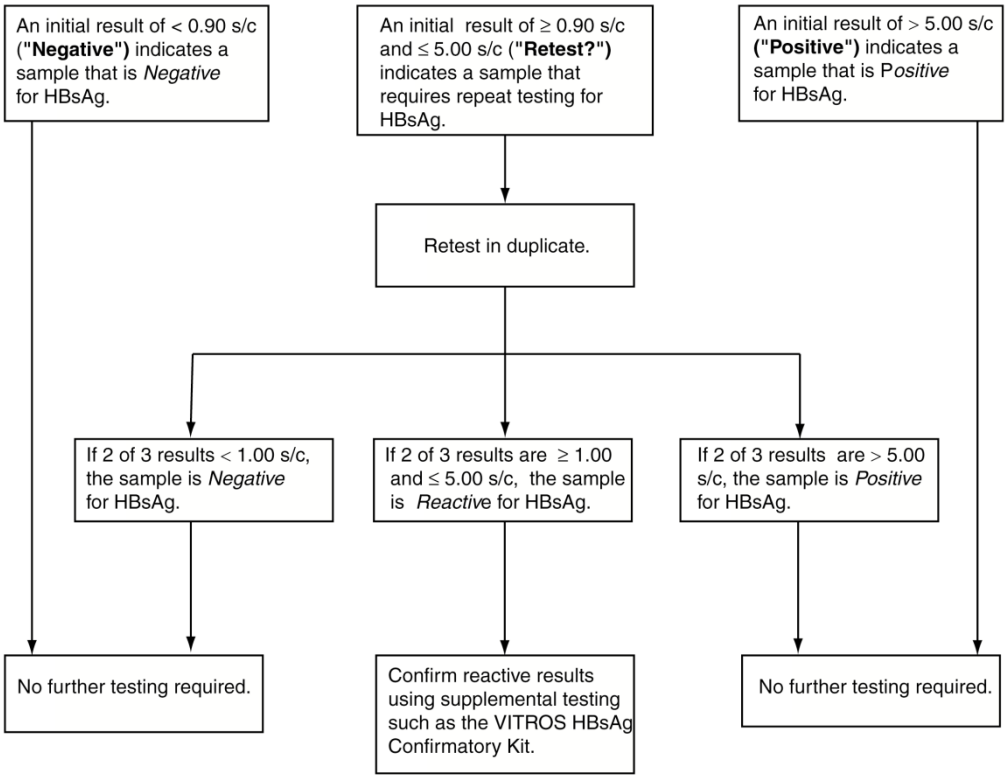
(1) Take off and discard screw caps from the cryo-vials and then load them in batches of 10 on the VITROS carousels. Ensure that the specimen ID barcode is readable in the holder’s window.
(2) Interface the Data Management System (DMS) with the VITROS instrument and submit the runsheet.

(3) Start the run and observe the transfer to make sure that all the specimens on the runsheet were scanned by the instrument before the test begins. If a barcode cannot be scanned due to incorrect positioning or an unreadable label, enter specimen ID manually.
(4) After completion of the test, interface DMS with the VITROS instrument and import the results into the DMS.

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system.

Testing Algorithm



e. Recording of Data

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems. The run information can be uploaded into the computerized database after the run information is exported from the software to the computerized database.

Result Calculation

Results are calculated as a normalized signal, relative to the cutoff value (signal/cutoff, s/c). During the calibration process, a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and Integrated Systems.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Signal at the cutoff (Cutoff value)}}$$

Patient sample results will be displayed with a **"Negative"**, **"Retest?"**, or **"Positive"** label. An initial result labeled with **"Retest?"** indicates a sample that requires repeat testing for HBsAg.

Result (s/c)	<0.90	≥0.90 and <5.00	>5.00
Result Text	**Negative**	**Retest?**	**Positive**

Final results should be manually interpreted using the algorithm below.

f. **Replacement and Periodic Maintenance of Key Components**

(1) Instruments are on service contract and except for the most basic daily maintenance are serviced by an Ortho Clinical Diagnostics technical representative.

Laboratory personnel monitor and document refrigerator temperature, freezer temperature, and room temperature on a daily basis

(2) All micropipettors used in testing clinical specimens are calibrated every 6 months. Pipettors that do not conform to specifications are autoclaved and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

g. **Calibrations**

Refer to the operating instructions for your system for detailed instructions on the calibration process.

h. Interpretation of results

The following table summarizes the interpretation of results obtained with the VITROS HBsAg test upon completion of all testing steps required in the testing algorithm.

Final VITROS HBsAg Test Result (s/c)	Conclusion from Testing Algorithm	Interpretation
<1.00	Negative	Specimen is presumed to be negative for HBsAg.
>1.00 and <5.00	Reactive	Specimen is reactive for HBsAg. If a reactive result is confirmed by supplemental tests, such as the VITROS Immunodiagnostic Products HBsAg Confirmatory Kit, the specimen is positive for HBsAg.
>5.00	Positive	Specimen is positive for HBsAg. *

* In instances where HBsAg is used as a standalone test (for example in pregnant women being screened to identify neonates who are at risk for acquiring HBV during the perinatal period), supplemental testing such as the VITROS HBsAg Confirmatory Kit should be used to confirm the result.

The magnitude of a VITROS HBsAg test result cannot be correlated to an endpoint titer.

The ability of the VITROS HBsAg test to detect HBV mutants has not been determined. Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

Heparin and citrate have been shown to lower the signal/cutoff (s/c) values in some HBsAg reactive samples. High negative results (0.80–0.99 s/c) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required.

9. REPORTABLE RANGE OF RESULTS

Final results are expressed qualitatively as positive or negative for the presence of HBsAg in the sample. No quantitative results are determined.

10. QUALITY CONTROL (QC) PROCEDURES

a. Quality Control Material Selection

VITROS HBsAg Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. There are 2 VITROS HBsAg Controls (a negative control and a HBsAg positive control). The performance of other commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other HBsAg methods if they contain high concentrations of preservatives, stabilizers, or other non-physiological additives, or otherwise depart from a true human sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS HBsAg test.

b. Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- The recommendation is to run a negative control and a positive control close to the HBsAg decision point (signal/cutoff $\geq$1.0).
- Choose control levels that check the clinically relevant concentrations.
- Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.
- To verify system performance, analyze control materials:
  - After calibration
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed or maintenance to critical parts or subsystems that might influence performance of the test

  If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.
- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to the published guidelines for general quality control recommendations.

For more detailed information, refer to the operating instructions for your system.

c. Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

a. If controls do not conform to specifications, reject the results and reanalyze all samples. Do not use data from no qualifying test runs.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. Known Interferences

The VITROS HBsAg test was evaluated for interference consistent with CLSI document EP7. Commonly encountered substances were tested on 2 lots of reagents. Of the compounds tested, none was found to interfere with the clinical interpretation of the test. Refer to "Substances that do not Interfere" for a list of compounds tested that did not show interference.

b. Other Limitations

- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture.
- Heterophilic, e.g. human anti- mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products.
- Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.
- HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with hepatitis B virus. Levels of HBsAg may be undetectable both in early infection and late after infection. In rare cases HBsAg tests do not detect certain HBV mutant strains.
- The analytical sensitivity of the VITROS HBsAg test was determined to be 0.085 IU/mL World Health Organization (WHO) 1st International Reference Standard 80/549), 0.030 PEI Units/mL (commercial ad subtype sensitivity panel), and 0.019 PEI Units/mL (commercial ay subtype sensitivity panel).
- Test performance characteristics have not been established for any other specimen matrices than serum or heparin, EDTA, and sodium citrate anticoagulated plasma.
- It has been shown that up to 498 µg HBsAg/mL does not create a high dose hook effect that will interfere with this test.
- Do not use quality control materials preserved with azide.

13. REFERENCE RANGES (NORMAL VALUES)

A normal human serum should be negative for hepatitis B surface antigens.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25 °C during preparation and testing for 4 hours.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other FDA-licensed tests for HBsAg may be substituted but must be accompanied by validation data to show substantial equivalence with these assays. Test methods may not be substituted without approval from NCHS.

Alternative methods of storage are not recommended. In case of system failure, samples should be refrigerated at 4-8°C for no more than 5 days. For longer periods, the specimens should be stored at - 20°C until the system is functioning properly.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database (Section 3) to track specimens.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data. For NHANES, residual specimens are stored frozen and returned to the NCHS specimen bank after testing for each cycle has been completed.

19. Summary Statistics and QC graphs

Qualitative assays are assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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