

# Laboratory Procedure Manual

*Analyte:*      **Hepatitis B Surface Antigen (HBsAg)**

*Matrix:*        **Serum**

*Method:*       **HBsAg VITROS Immunodiagnostic Products (Ref 680 1322)**

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*As performed by:*    **Diagnostic Reference Team  
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## **Important Information for Users**

The National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention 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:

<b>Data File Name</b>	<b>Variable Name</b>	<b>SAS Label</b>
HEPBD_K	LBDHBG	Hepatitis B surface antigen

## 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical relevance:

Hepatitis is inflammation of the liver most often caused by a virus. Viral hepatitis is a major public health problem of global importance because of the ongoing transmission of viruses that cause the disease and increased morbidity and mortality associated with the acute and chronic consequences of these infections. Global and US goals have been established for elimination of viral hepatitis as a public health threat by 2030.

In the US, the most common types of viral hepatitis are hepatitis A, B, and C. Effective vaccines are available to help prevent hepatitis A and hepatitis B. No vaccine is available for hepatitis C; however, highly effective, well-tolerated treatment can cure hepatitis C virus infection. Hepatitis D virus infection is less common in the US and can occur only among persons with hepatitis B virus infection. Hepatitis E infection also is less common in the US. These five hepatitis viruses, also called hepatitides, are well-characterized for detection with laboratory assays and are monitored in U.S. public health surveillance systems.

NHANES viral hepatitis data are used to monitor progress toward goals in *Healthy People* and the HHS *Viral Hepatitis National Strategic Plan*, which in turn support US and global viral hepatitis elimination goals. The viral hepatitis laboratory and interview components of NHANES complement data from outbreak, case-based surveillance, vital statistics, health care systems, and cohort studies that can provide timely, detailed, or longitudinal information for subnational geographic areas and disproportionately affected populations (such as persons experiencing homelessness or living in correctional facilities); however, these sources lack information available from NHANES, such as race, ethnicity, education, income, and health status and behaviors.

Viral hepatitis data from NHANES are available beginning with the Second NHANES conducted during 1976-1980 for hepatitis A and hepatitis B, and with the Third NHANES conducted during 1988-1994 for hepatitis C, hepatitis D and hepatitis E.

An estimated 300 million people worldwide are persistent carriers of hepatitis B virus (HBV). Infection with HBV results in a wide spectrum of acute and chronic liver diseases that may lead to cirrhosis and hepatocellular carcinoma. Co-infection with hepatitis D virus (HDV) in persons with acute or chronic hepatitis B virus (HBV) infection can lead to fulminant hepatitis.

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.

Anti-HBc is detectable shortly after the appearance of hepatitis B surface antigen (HBsAg). As the appearance of anti-HBsAg may be delayed after HBsAg clearance, anti-HBc is sometimes the only serological marker for HBV infection and potentially infectious blood. Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved 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.

The Delta antigen/antibody system (HDAg/Anti-HD) is related to HBV infection but immunologically distinct from its known reactivities; it is the expression of the Delta virus (HDV. Hepatitis D Virus), a cause of severe liver disease in HBsAg carriers. HDV is a 35-37nm particle containing low molecular weight RNA and HDAg, with an outer coat of HBsAg obtained from HBV. HDV is a defective virus and its replication requires helper functions provided by HBV. HDAg has been detected in liver and in serum and induces a specific antibody response (anti-HD antibodies) in both the IgG and IgM classes.

The NHANES viral hepatitis laboratory component tests for anti-HBc, HBsAg among anti-HBc positive specimens, and anti-HDV among HBsAg positive specimens.

Examined participants aged 6 years and older in the NHANES 2019-March 2020 sample were eligible for the anti-HBc, HBsAg, and anti-HDV tests.

#### Test principle:

Hepatitis B surface antigen is measured using the VITROS HBsAg test, which 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.

## 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

The Data Management System (DMS) was used through December 31, 2019.

The run information can be uploaded into the computerized database (DMS) after the run information is exported by the software. This database was custom-designed for the management of CDC Division of Viral Hepatitis (DVH) Laboratory Branch (LB) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. In August 2019, laboratory data management was transferred to the CDC Enterprise Laboratory Information System (ELIMS), where NHANES functionality was reproduced and improved over time to include more process automation. DMS was maintained in parallel until December 31, 2019, when it was discontinued. Finished DMS data were reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. Files stored on the CDC Local Area Network (LAN) were automatically backed up nightly by CDC Data Center staff.

Documentation for data system maintenance was maintained with printed copies of data records for 2 years.

CDC Enterprise Laboratory Information System (ELIMS) is has been used since January 1, 2020, for accessioning, test results processing, reporting and storage. Finished ELIMS data are reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. All information about the accessioned specimens, traceability of the diagnostic process, test runs and reported results are stored in the ELIMS database, are archived after 12 months and can be retrieved any time upon request.

Documentation for ELIMS is maintained on an intranet site accessible by laboratory staff.

#### **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  $-20^{\circ}\text{C}$  ( $-4^{\circ}\text{F}$ ).

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

Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29-A. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

## **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. Instrumentation and Software**

- VITROS ECi/ECiQ Immunodiagnostic Systems
- VITROS 3600 Immunodiagnostic System

### **b. 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

### **c. Materials Provided**

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

### **d. 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.

#### e. 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.

#### f. Preparation of Quality Control Material

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



## g. 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 (36–46°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.

#### **h. 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 (36-46°F); 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 [59-86 °F]).

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

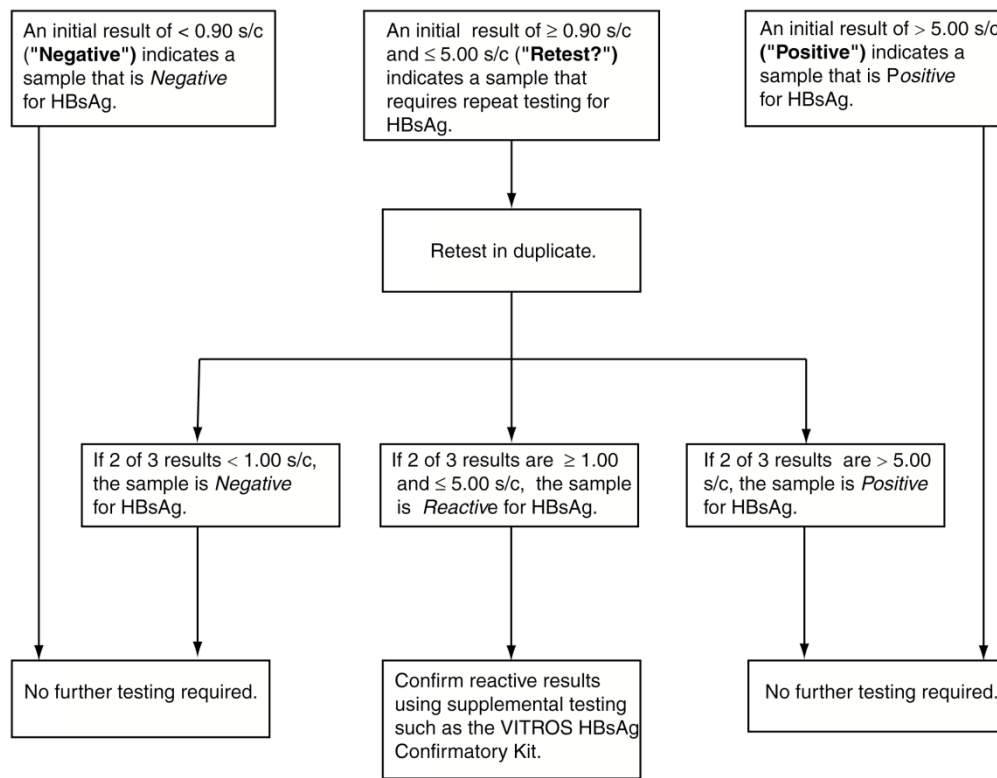
- (1) Test procedure is performed as described in VITROS 3600 Operation and Maintenance Procedure and VITROS® Anti-HBc Total (aHBc) Technical Procedure using the VITROS Anti-HBc Reagent Pack and associated controls and calibrator packs. Do not use expired reagents.
- (2) During the use of the Data Management System:
  - a. 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.
  - b. Interface the Data Management System (DMS) with the VITROS instrument and submit the runsheet.
  - c. 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.
  - d. After completion of the test, interface DMS with the VITROS instrument and import the results into the DMS.
- (3) During the use of the Enterprise Laboratory Information Management System (ELIMS):
  - a. The specimens arriving to CDC are accessioned by the Specimen Triage and Tracking Team (STATT) into ELIMS for Unit 90 under CLIA regulated practices, and then Unit 90 is notified.
  - b. Testing Personnel within Unit 90 pick-up specimens within 24 hours of notice.
  - c. DRT TP transports the specimens to DRT Pre-analytic testing facilities. Specimens are securely stored ensuring conditions described in the Office of the Associate Director for Laboratory Science and Safety (OADLSS) Biosafety Manual.
  - d. Process specimens in ELIMS by following the Pre-Analytic Workflow of the ELIMS Job Aids for User Level 2 Data Manager.
  - e. Fill out the VITROS Runsheet.
  - f. Before testing, samples in cryovials will be centrifuged at 5000 g for 5 minutes.
  - g. Upon completion of centrifugation, cryovials shall be open and all the caps shall be disposed.
  - h. Place cryovials in the VITROS trays, up to 10 vials at a time.
  - i. Once the results are generated by the VITROS 3600, download the data into ELIMS according to the Post-Analytic Workflow of the ELIMS Job Aid for User Level 2 Data Manager.
  - j. Once completed, notify the supervisor.

#### d. Operation of Assay Procedure

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.

#### e. Testing Algorithm



#### f. Recording of Data

##### (1) Quality Control Data

Quality control is measured using manufacturer provided positive and negative controls.

VITROS HBsAg Negative Control should generate Non-reactive results.

VITROS HBsAg Positive Control should generate Reactive results.

Only runs that pass with controls within the designated range are reported from the analyzer.

If controls fail or are outside of acceptable range, no results can be generated from the analyzer until controls fall within range.

Raw data are processed by the VITROS Immunodiagnostics System and transferred automatically from the VITROS instrument into the DMS or ELIMS.

#### g. 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.

#### h. 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 removed from the workflow, disinfected and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

#### i. Calibrations

Calibration and maintenance procedures/requirements for each piece of equipment can be found in procedure VITROS 3600 Operation and Maintenance Standard Operating Procedure.

Calibration is lot-specific; reagent packs and calibrator 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.

The lot-specific parameter [a] links the signal at the cutoff (cutoff value) to the calibrator signal:

Cutoff value = (a X Signal of Cal 1)

Process calibrator in the same manner as samples. Load sufficient calibrator for the automatic duplicate determination. Calibration does not need to be programmed if barcoded labels are used; calibration will be initiated automatically.

When the calibrator is processed, the validity of the calibration is assessed against quality parameters which compare 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 calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.

#### **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 acceptable range

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

#### **Traceability of Calibration**

The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.

#### **Calibration Model**

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

#### **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.

<b>Final VITROS HBsAg Test Result (s/c)</b>	<b>Conclusion from Testing Algorithm</b>	<b>Interpretation</b>
<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.

### Test Limit of Detection

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.

## 10. QUALITY CONTROL (QC) PROCEDURES

### a. Quality Control Material Selection

Quality control is measured using manufacturer provided positive and negative controls.

VITROS HBsAg Negative Control should generate Non-reactive results.

VITROS HBsAg Positive Control should generate Reactive results.

Only runs that pass with controls within the designated range are reported from the analyzer.

### ***Positive Controls***

Control	Frequency (every run, every extraction, etc.)	Expected Value
VITROS HBsAg Positive Control	After calibration; At least once each day that the test is being performed; After specified service procedure or maintenance to critical parts or subsystems that might influence performance of the test.	Positive

### ***Negative Controls***

Control	Frequency (every run, every extraction, etc.)	Expected Value
VITROS HBsAg Negative Control	After calibration; At least once each day that the test is being performed; After specified service procedure or maintenance to critical parts or subsystems that might influence performance of the test.	Negative

### ***Additional Controls***

Control	Frequency (every run, every extraction, etc.)	Expected Value
None	Not applicable	Not applicable

### ***Quality Control Procedures***

Controls that fall outside the acceptable range should result in an immediate retest.

If a second failure results, refer to VITROS 3600 Operation and Maintenance Manual.

If a third failure results, call Ortho CDC VITROS technical support line to troubleshoot either by phone, or schedule field service visit.

If failure occurs 3 times, TP submits a nonconforming event report describing the sequence of occurrences.

**NOTE:** No specimens are tested or results generated from the analyzer until controls fall within the acceptable range.

#### **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 non-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) 1<sup>st</sup> 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.

#### **c. Substances that do not interfere**

As recommended by NCCLS Protocol EP7, the VITROS HBsAg test was evaluated for interference by testing the substances listed in the table below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with HBsAg at a target s/c of  $2.00 \pm 1.00$  with two lots of reagent. None of the compounds at the levels tested were found to interfere with the clinical interpretation of the test.

The following compounds were tested at the levels listed:

Bilirubin, 0.35 mmol/L

Hemoglobin, 0.31 mmol/L

Triolein, 33.9 mmol/L

### **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 (68–77 °F) 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 (39–46 °F) for no more than 48 hours. For longer periods, the specimens should be stored at - 20°C (-4 °F) or below until the system is functioning properly, and returned to the NCHS specimen bank after testing for each cycle has been completed

## **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. The controls are read as cutoff values. However, QC numeric data are monitored over time using Quality Control Charts to detect trends or shifts in performance through regular, planned reviews by supervisors, i.e., monthly, or quarterly. Reviews are used to identify opportunities for improvements.

Evaluation of the Quality Control of Test Results on VITROS Immunodiagnostic System is conducted according to the internal laboratory Standard Operating Procedure, available to the laboratory personnel.

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