

## Laboratory Procedure Manual

*Analyte:* **Hepatitis B Surface Antibody (anti-HBS)**

*Matrix:* **Serum**

*Method:* *aHBs-Anti-HBs*  
**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, 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>
HEPB_S_H	LBXHBS	Hepatitis B Surface Antibody (anti-HBs)

## 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The VITROS Anti-HBs test is performed using the VITROS Anti-HBs Reagent Pack and the VITROS Anti-HBs Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System using Intellicheck<sup>®</sup> Technology. An immunometric immunoassay technique is used. This involves the reaction of anti-HBs present in the sample with an HBsAg (ad and ay subtypes) coated on the wells. The horseradish peroxidase (HRP)-labeled HBsAg conjugate (ad and ay subtypes) then complexes with the bound anti-HBs forming an "antigen sandwich". Unbound materials are 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 directly proportional to the concentration of anti-HBs present.

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. Testing for hepatitis B surface antibody contributes information to distinguish immunity from vaccination among persons who lack antibodies to hepatitis B core antigen. Assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection for individuals prior to or following HBV vaccination, or where vaccination status is unknown

## 2. SAFETY PRECAUTIONS

All reagents included in the kit are intended for "in vitro diagnostic use".

Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.

Do not pipette by mouth.

Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.

Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.

Avoid spilling samples or solutions containing samples.

Provide adequate ventilation.

*The VITROS Anti-HBS conjugate reagent and assay reagent 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 MANAGEMENT SYSTEM**

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. Reporting is done directly from the database in printed form or by electronic transfer.

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

Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly to tape by CDC Data Center staff.

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

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

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

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surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

No special patient preparation is necessary.

*Specimens Recommended: Serum*

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

Do not use plasma samples.

Collect specimens using standard procedures.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result.

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

The VITROS Anti-HBs 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 samples in stoppered containers to avoid 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 the system prior to analysis should be limited to avoid evaporation. This time should not exceed 2 hours. Refer to the operating instructions for your system.

Return to 2–8 °C (36–46 °F) as soon as possible after use, or load sufficient volume for a single determination.

The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing serum 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 serum at 2–8 °C (36–46 °F).
- If the test will not be completed within 48 hours, or for shipment, freeze the serum 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. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION**

**a. Instrumentation and Software**

VITROS ECI/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System

**b. Reagents**

*Reagent Pack Contents*

1 reagent pack containing:

- 100 human HBsAg (ad and ay subtypes) coated wells
- 13.3 mL conjugate reagent: human HBsAg (ad and ay subtypes)-HRP conjugate in phosphate buffered saline with human plasma, protein stabilizers and antimicrobial agent (Kathon, 2% w/v)
- 6.2 mL assay reagent: EDTA phosphate buffered saline with 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 on 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*

<b>Reagent</b>	<b>Storage Condition</b>		<b>Stability</b>
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 Anti-HBs Quantitative 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.

c. *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 quantitative *in vitro* determination of total antibody to hepatitis B surface antigen (anti-HBs) in human serum using the VITROS Immunodiagnostic Products Anti-HBs Quantitative Reagent Pack.

*Calibrator Contents*

- 1 set of VITROS Anti-HBs Calibrators 1, 2 and 3 (re-calcified human plasma with antimicrobial agent, 2.0 mL, Bronidox 1.0%); nominal values 0; 30 and 250 mIU/mL
- Lot calibration card
- Protocol card
- 24 calibrator bar code labels (8 for each calibrator)

*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 VITROS Immunodiagnostic and VITROS Integrated Systems. 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*

<b>Calibrator</b>	<b>Storage Condition</b>		<b>Stability</b>
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	13 weeks
Opened	Frozen	-20 °C (-4 °F)	13 weeks

- VITROS Anti-HBs Calibrators are supplied ready for use.
- The VITROS Anti-HBs Calibrators are 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 Anti-HBs test uses 80 µL of calibrator for each determination. The VITROS Anti-HBs 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.

d. Materials Provided

- VITROS Immunodiagnostic Products Anti-HBs Quantitative Reagent Pack
- VITROS Immunodiagnostic Products Anti-HBs Calibrators

e. Materials Required but not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- VITROS Immunodiagnostic Products High Sample Diluent B Reagent Pack
- Quality control materials such as VITROS Immunodiagnostic Products Anti-HBs Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

## 7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

### a. Calibration

#### *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 (a dose response curve covering the full calibration range) is established for each new reagent lot. Concentrations for the linked lot of calibrators are determined from the Master Calibration.
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.
- When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration curve is assessed against a range of quality parameters, and if acceptable, it is 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 range of quality parameters. Failure to meet any of the defined quality parameter ranges 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.

#### *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.

#### *Traceability of Calibration*

The calibration of the VITROS Anti-HBs Quantitative test is traceable to the World Health Organization (WHO) First International Reference Preparation for Antibody to HBsAg (1977).

#### *Calibration Model*

A modified four-parameter logistic curve fit function is used to construct the Master Calibration. The calibration process rescales the Master Calibration to establish a valid stored curve for the VITROS Immunodiagnostic and VITROS Integrated Systems.

### **b. Verification**

Not Applicable

## **8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

### **a. Preliminaries**

- (1) The VITROS aHBs – Anti-HBs Reagent Pack is used for 100 tests. Reagent pack is supplied ready for use and its 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 packs is loaded on the instrument directly from refrigerated storage to minimize condensation
- (4) Prepare a run sheet listing controls and specimens in the order presented in the e-file.
- (5) Perform daily maintenance of the VITROS ECi and V3600 instruments according to user manual; verify the validity of the calibrators and if needed update. Run negative and positive controls.

b. Sample Preparation

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

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

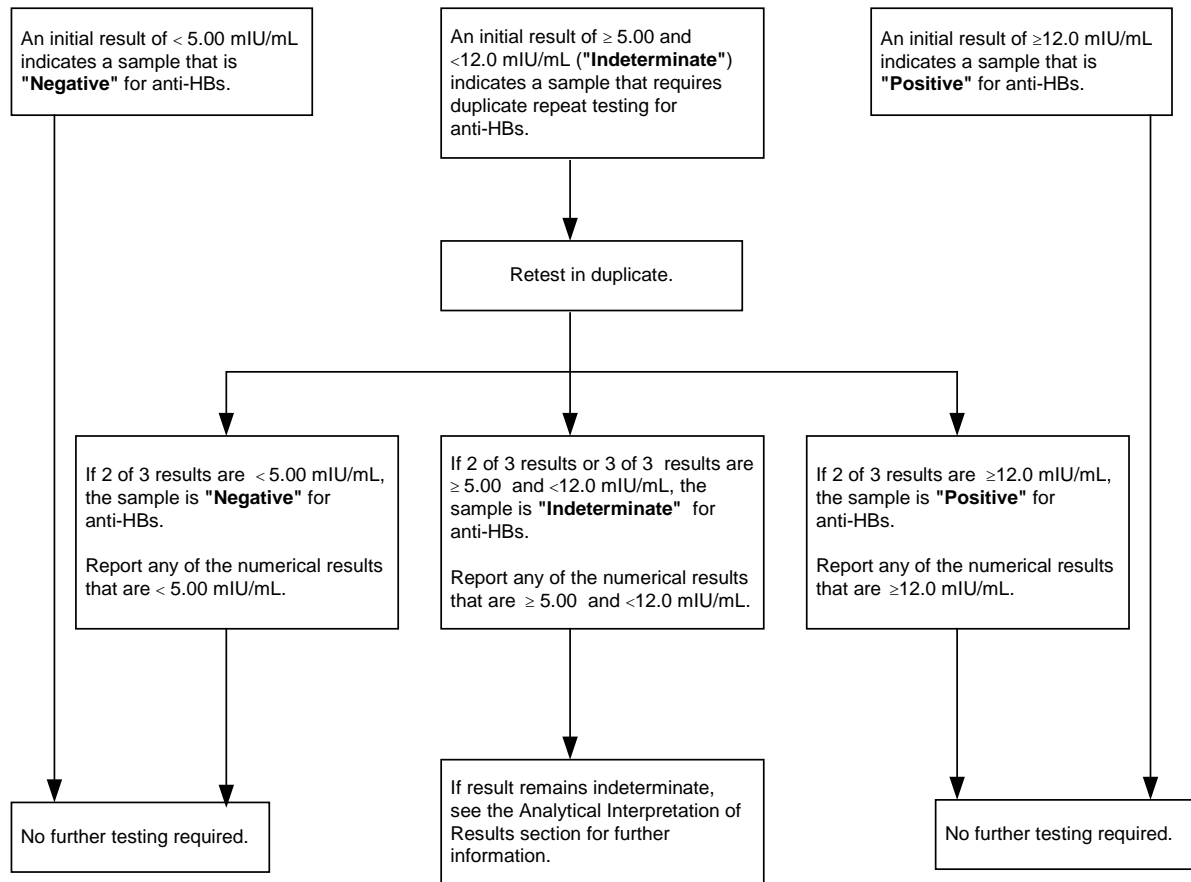
- (1) Take off and discard screw caps from the cryo-vials, than load them in batches of 10 on the VITROS carousels. Ensure that the specimen ID barcode is readable in the holder's window.
- (2) Interface the Data Management System (DMS) with the VITROS instrument and submit the run sheet.
- (3) Start the run and observe the transfer to make sure that all the specimens on the run sheet were scanned by the instrument before the test begins. If a barcode cannot be scanned due to incorrect positioning or an unreadable label, enter the 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

Final results should be manually interpreted using the algorithm below.



#### d. Reporting results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

##### *Reporting Units and Unit Conversion*

Analyte results are quoted in units of mIU/mL. To configure the units, refer to the operating instructions for your system.

#### e. Interpretation of Results

##### Analytical Interpretation

- A result of  $< 4.23\text{ mIU/mL}$  indicates that the result is below the test's Limit of Detection (LoD). The sample is "Negative" for anti-HBs and the individual is not immune to HBV infection.
- A result of  $\ge 4.23\text{ mIU/mL}$  and  $< 5.00\text{ mIU/mL}$  indicates with 95% confidence that a sample contains anti-HBs, but not at levels consistent with protective immunity against HBV infection.

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- A result of  $\geq 12.0$  mIU/mL indicates that a sample is “Positive” for anti-HBs. This result is consistent with levels of anti-HBs at  $>10$  mIU/mL, which indicates that anti-HBs has been detected at levels consistent with protective immunity against HBV infection.
- A specimen with a result of  $\geq 5.00$  mIU/mL and  $<12.0$  mIU/mL indicates that a sample is “Indeterminate” for anti-HBs and should be retested in duplicate. If both repeats are  $<5.00$  mIU/mL, the specimen is negative for anti-HBs. If both repeats are  $\geq 12.0$  mIU/mL, the specimen is positive for anti-HBs. The result is indeterminate if one or both replicate results are  $\geq 5.00$  mIU/mL and  $<12.0$  mIU/mL. If a result remains indeterminate, the immune status of the individual should be further assessed by considering other factors, such as clinical status, follow-up testing, associated risk factors, and the use of additional diagnostic information.
- Results obtained with the VITROS Anti-HBs Quantitative test may not be used interchangeably with values obtained with different manufacturers’ test methods.

Clinical Interpretation

VITROS Anti-HBs Test Result	Result Text	Clinical Interpretation of Immune Status
$<5.00$ mIU/mL	Negative	Patient is considered to be not immune to infection with HBV.
$\geq 5.00$ mIU/mL and $<12.0$ mIU/mL	Indeterminate	Unable to determine if anti-HBs are present at levels consistent with immunity. Patient's immune status should be further assessed by considering other clinical information or retesting another specimen drawn at a later time.
$\geq 12.0$ mIU/mL	Positive	Anti-HBs detected at $>10$ mIU/mL. Patient is considered to be immune to infection with HBV. It has not been determined what the clinical significance is for values greater than $>12$ mIU/mL, other than the individual is considered to be immune to HBV infection.

f. Recording of Data

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems. Data stored by the VITROS Immunodiagnostic and VITROS Integrated Systems are uploaded to the DMS for review.

g. Calculations

Not Applicable

## 9. REPORTABLE RANGE OF RESULTS

Final results are expressed qualitatively as positive or negative for the presence of anti-HBs antibody in the sample. No quantitative results are reported for NHANES.

System	Measuring (Reportable) Range
ECi/ECiQ, 3600	4.23*–1000 mIU/mL

\* Lower limit of measuring range is based on the Limit of Detection. The lowest result reported by the system software is 0 mIU/mL. Values between 0 and 4.23 mIU/mL should be interpreted as not having detectable anti-HBs.

### ***Test Limit of Detection***

The lowest amount of anti-HBs that can be detected with the VITROS Anti-HBs Quantitative test was determined in accordance with NCCLS EP17. Based upon 274 positive determinations, the Limit of Detection (LoD) is 4.23 mIU/mL of anti-HBs, with a 95% probability of obtaining a measurable response at that level. A Limit of Blank (LoB) of 3.08 mIU/mL was used.

## 10. QUALITY CONTROL (QC) PROCEDURES

### *Quality Control Material Selection*

VITROS Anti-HBs Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. The performance of other commercial control fluids should be evaluated for compatibility with this test before they are used for quality control. Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Anti-HBs test.

Choose control material that has the composition of the patient sample matrix being analyzed.

### *Quality Control Procedure Recommendations*

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- Choose control levels that check the clinically relevant concentrations. The recommendation is to run a negative control and a positive control close to the anti-HBs decision point (10 mIU/mL)
- 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 or maintenance to critical parts or subsystems that might influence the performance of the test
  - Analyze quality control materials in the same manner as patient specimens.
  - If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within the acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
  - \*Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control recommendations.
  - Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- For more detailed information, refer to the operating instructions for your system.

#### *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**

The entire run is considered to be invalid if one or both controls are not within specified limits. If **Negative Control, or Positive Control** is invalid then the entire run is invalid, repeat the entire run including control and sample preparation.

## **12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS**

- Test performance characteristics have not been established when the VITROS Anti-HBs Quantitative test is used in conjunction with other manufacturer's tests for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Test performance characteristics have not been established for the use of the VITROS Anti-HBs Quantitative test as an aid in determining susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.
- The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.
- This test does not differentiate between a vaccine induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc test may be performed.

- Individuals that have received blood component therapy, e.g., whole blood, plasma, immune globulin administration, during the previous 3–6 months may have a false reactive anti-HBs result due to passive transfer of anti-HBs.
- Certain drugs and clinical conditions are known to alter anti-HBs concentrations *in vivo*. For additional information, refer to one of the published summaries.
- Results from immune-suppressed individuals should be interpreted with caution.
- Individuals possessing IgM anti-rubella virus may have falsely high results with the VITROS Anti-HBs Quantitative test.
- Test performance characteristics have not been established for any other specimen matrix than serum.
- The prevalence of the analyte will affect the test's predictive value.
- Turbidity may affect test results.

### **13. REFERENCE RANGES (NORMAL VALUES)**

A normal human serum should be negative for hepatitis B surface antibodies unless the individual has been vaccinated against hepatitis B.

### **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 only.

### **16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS**

Other FDA-licensed tests for Hepatitis B surface antibody may be substituted but must be accompanied by validation data to show substantial equivalence with this assay. Substitution of test methods may not be done without approval from the NCHS.

Alternate storage is not recommended.

### **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 that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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