

Laboratory Procedure Manual

Analyte: **Hepatitis B Core Antibody**

Matrix: **Serum**

Method: **aHBc – Anti-HBc**
VITROS Immunodiagnostic Products (REF 680 1428)

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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:

Data File Name	Variable Name	SAS Label
HEPBD_K	LBXHBC	Hepatitis B core antibody

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 behavior.

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.

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 core antibody is measured using the VITROS Anti-HBc test, which is performed using the VITROS Anti-HBc Reagent Pack and VITROS Immunodiagnostic Products Anti-HBc Calibrator on the VITROS ECi/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System.

A competitive immunoassay technique is used. This involves the reaction of anti-HBc in the sample with hepatitis B core antigen (HBcAg) coated wells. Unbound sample is removed by washing. Horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-HBc) is then allowed to react with the remaining exposed HBcAg on the well surface. 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 concentration of anti-HBc present.

The VITROS Anti-HBc test can be used to detect antibodies against hepatitis B core antigen (anti-HBc) in serum and plasma following exposure to infectious hepatitis B virus (HBV). 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.

2. SAFETY PRECAUTIONS:

Test kits for anti-HBc 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 ANTI-HBc 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 through 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) 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

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- EDTA plasma
- Citrate plasma

Specimens Not Recommended

- Do not use turbid specimens. Turbidity in specimens may affect test results.
- Do not use heat inactivated samples.

Specimen Collection and Preparation

- 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 Anti-HBc test uses 50 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.

Handling and Storage Conditions

- 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 the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the operating instructions for your system.
- The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing 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 samples at 2–8°C (36–46°F).
 - If the test will not be completed within 48 hours, or for shipment, freeze samples 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.
- 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 Anti-HBc Controls
- VITROS Immunodiagnostic Products High Sample Diluent B
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

c. Materials Provided

VITROS Immunodiagnostic Products Anti-HBc Reagent Pack
VITROS Immunodiagnostic Products Anti-HBc Calibrator

d. Reagent Preparation

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells [recombinant HBcAg derived from bacteria (*E.coli*) coated at 1.5 ng per well]
- 14.6 mL assay reagent [buffer with newborn calf serum, bovine gamma globulins and antimicrobial agent (0.5% Kathon w/w)]
- 20.6 mL conjugate reagent (HRP-mouse monoclonal anti-HBc, 0.1 µg/mL), in buffer with mouse serum, human plasma and antimicrobial agent (0.5% Kathon w/w)

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 Anti-HBc 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

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 Systems.

f. **Preparation of Quality Control Material**

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 and the VITROS 3600 Immunodiagnostic System for the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis B core antigen (total anti-HBc) in human adult and pediatric serum and plasma (EDTA and citrate) and neonate serum using VITROS Anti-HBc Reagent Packs.

h. **Calibrator Contents**

- 1 VITROS Anti-HBc Calibrator (anti-HBc negative human plasma, 2.2 mL) with antimicrobial agent, Bronidox 1.0%
- Lot calibration card
- Protocol card
- 8 calibrator bar code labels

i. 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.

j. Calibrator Storage and Preparation

Calibrator	Storage Condition	Stability
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

- The VITROS Anti-HBc Calibrator is supplied ready for use.
- The VITROS Anti-HBc Calibrator is suitable for use until the expiration date on the carton when they are 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-HBc test uses 50 µL of calibrator for each determination. The VITROS Anti-HBc 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 Anti-HBc Calibrator is automatically processed in duplicate.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

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 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-HBc test is traceable to in-house reference calibrators, which have been value-assigned to optimize the clinical sensitivity and specificity performance.

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 aHBc 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 (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; 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) (59–86 °F) before using.

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, 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 the 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 at 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 (TP) 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. Recording of Data

(1) Quality Control Data

- Quality control is measured using manufacturer provided positive and negative controls.
- VITROS Anti-HBc Negative Control should generate Non-reactive results.
- VITROS Anti-HBc Positive Control should generate Reactive results.

- Only runs that pass with controls within the designated range are reported from the analyzer.
- Controls that fall outside the acceptable range should result in an immediate retest.
- If controls fail and are outside of acceptable range, no specimens are tested on the analyzer until controls fall within range.
- Raw data are processed by the VITROS Immunodiagnosics System and transferred automatically from the VITROS instrument into the DMS or ELIMS.

(2) Result Calculation

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

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 VITROS Integrated Systems.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

Patient sample results will be displayed with a **“Reactive”**, **“Retest?”**, **“Negative”** or **“Equivocal”** label. An initial result labeled with **“Retest?”** indicates a sample that requires duplicate repeat testing for anti-HBc. An initial result labeled **“Equivocal”** indicates a sample that requires dilution and re-tests.

Result (s/c)	<0.90	≥0.90 and ≤1.10	>1.10 and <4.80	≥4.80
Result Text	Reactive	Retest?	Negative	Equivocal

f. Replacement and Periodic Maintenance of Key Components

- (1) Instruments are on service contract and except for the routine daily, weekly, and monthly maintenance are serviced by an Ortho Clinical Diagnostics technical representative.

Laboratory personnel monitor and document refrigerator temperature, freezer temperature, and room temperature daily.

- (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 pipettors 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 Anti-HBc test upon completion of all testing steps required in the testing algorithm.

Initial VITROS Anti-HBc Test Result (s/c)	Conclusion from Testing Algorithm	Interpretation
<0.90	Reactive	Specimen is presumed to be reactive for anti-HBc.
≥0.90 and ≤1.10	Retest in duplicate	If 2 of 3 results are <1.00 then specimen is presumed to be reactive for anti-HBc
≥0.90 and ≤1.10	Retest in duplicate	If 2 of 3 results are >1.00 and <4.80 then specimen is negative for anti-HBc.
>1.10 and <4.80	Negative	Specimen is negative for anti-HBc.
≥4.80	Dilute 1:20 and retest	If 1:20 dilution and retest* result is <1.00, then specimen is presumed to be reactive for anti-HBc.
≥4.80	Dilute 1:20 and retest	If 1:20 dilution and retest* result is >1.00 and <4.80, then specimen is negative for anti-HBc.

* Results of the diluted sample do not require correction for the dilution factor.

- Results obtained with the VITROS Anti-HBc test may not be used interchangeably with values obtained with different manufacturers' test methods.
- The magnitude of a VITROS Anti-HBc test result cannot be correlated to an endpoint titer.
- Neonate samples with results ≥0.90 and ≤1.10 should not be retested in duplicate. Obtain a new sample and retest.

9. REPORTABLE RANGE OF RESULTS

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

10. QUALITY CONTROL (QC) PROCEDURES

Quality Control Material Selection

Quality control is measured using manufacturer provided positive and negative controls. VITROS Anti-HBc Negative Control should generate Non-reactive results. VITROS Anti-HBc Positive Control should generate Reactive results.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Anti-HBc test. Only runs that pass with controls within the designated range are reported from the analyzer.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.

Positive Controls

Control	Frequency (every run, every extraction, etc.)	Expected Value
VITROS Anti-HBc 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 Anti-HBc 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

- 1.1.1 Controls that fall outside the acceptable range should result in an immediate retest.
 - 1.1.2 If a second failure results, refer to VITROS 3600 Operation and Maintenance Manual.
 - 1.1.3 If a third failure results, call Ortho CDC VITROS technical support line to troubleshoot either by phone, or schedule field service visit.
 - 1.1.4 If failure occurs subsequently 3 times, TP submits a nonconforming event report describing the sequence of occurrences.
- **NOTE:** No specimens are tested or results are generated from the analyzer until controls are within the acceptable range.

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

Known Interferences

The VITROS Anti-HBc 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.

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.
- A negative test result does not exclude the possibility of exposure to hepatitis B virus. Levels of anti-HBc may be undetectable both in early infection and late after infection.

- Heterophilic antibodies in serum or plasma samples may 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. Results, which are inconsistent with clinical observations, indicate the need for additional testing.

Substances that do not Interfere

The VITROS Anti-HBc test was evaluated for interference. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with anti-HBc to level near the s/c of 2.00. None of the compounds at the levels tested were found to interfere with the clinical interpretation of the test. Similarly, no interference was observed in samples not spiked with anti-HBc (Negative), with anti-HBc values remaining above 2.00 s/c.

The following compounds were tested at the levels listed:

- a) Bilirubin, 20 mg/dL
- b) Hemoglobin, 500 mg/dL
- c) Triolein, 3000 mg/dL

13. REFERENCE RANGES (NORMAL VALUES)

The reference value for Anti-HBc is negative in a healthy, unexposed population.

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 total anti-HBc core antibody 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) 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 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. 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.

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