

Laboratory Procedure Manual

Analyte: **Hepatitis B Core Antibody**
Matrix: **Serum**
Method: **aHBc – Anti-HBc**
 VITROS Immunodiagnostic Products (REF 680 1428)
Method No.:

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

The National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP) periodically refines this 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, has been incorporated.

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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_H | LBXHBC | Hepatitis B core antibody |

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The VITROS Anti-HBc test 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

- a. The run information can be uploaded into the computerized database after the run information is exported by the software to the computerized database. This database was custom-designed for the management of CDC Assay Development and Diagnostic Reference Laboratory (ADDRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. Test values are compared with a cutoff value calculated from controls. Results are expressed as "positive" or "negative" for anti-HBc. Other information in the database may typically include the ADDRL identification number, the specimen number, the date collected, the date tested, and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer.
- b. Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with the other NHANES IV data.
- c. Files stored on the LAN are automatically backed up nightly by CDC Data Center staff.
- d. Documentation for data system maintenance is contained in hard copies of data records for 2 years.

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

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.

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- 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.

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

Not applicable for this procedure.

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

a. Required Materials not Provided

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

b. Materials Provided

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

c. 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

d. Standards Preparation

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

e. Preparation of Quality Control Material

- (1) Kit positive and negative controls are prepared and quality controlled by the manufacturer.
- (2) In-house controls are prepared according to ADDRL specifications.

f. Calibrators

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System for the *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.

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

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 | 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; 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) 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) 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 runsheet.
- (3) Start the run and observe the transfer to make sure that all the specimens on the runsheet were scanned by the instrument before the test begins. If a barcode cannot be scanned due to incorrect positioning or an unreadable label, enter 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.

d. Recording of Data

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

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 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 |

e. 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 on a daily basis

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

f. Calibrations

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

g. 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

VITROS Anti-HBc 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.

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.

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

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

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-HBc decision point [signal/cutoff (s/c) ≤ 1.00].
- 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 performance of the test

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.

- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
- 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

- 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.

13. REFERENCE RANGES (NORMAL VALUES)

A normal human serum should be negative for hepatitis B core antibodies.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

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

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

Other FDA-licensed tests for 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 for no more than 5 days. For longer periods, the specimens should be stored at - 20°C until the system is functioning properly.

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

Not applicable

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

Test results are documented through the lab management database (Section 3) to track specimens. Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data. For NHANES, residual specimens are stored frozen and returned to the NCHS specimen bank after testing for each cycle has been completed.

19. Summary Statistics and QC graphs

Qualitative assays are assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value 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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