Laboratory Procedure Manual

Analyte: Hepatitis B Core Antibody
Matrix: Serum
Method: aHBc – Anti-HBc
VITROS Immunodiagnostic Products (REF 680 1428)

Method No.: 

First Published: February 24, 2011
Revised: N/A

As performed by: Assay Development and Diagnostic Reference Laboratory (ADDRL)
Laboratory Branch
Division of Viral Hepatitis
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

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

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

<table>
<thead>
<tr>
<th>Data File Name</th>
<th>Variable Name</th>
<th>SAS Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPBD_F</td>
<td>LBXHBC</td>
<td>Hepatitis C core antibody</td>
</tr>
</tbody>
</table>
INSTRUCTIONS FOR USE aHBc

VITROS Immunodiagnostic Products

Anti-HBc Reagent Pack

REF 680 1428

Version 3.0 Pub. No. J20866

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

Intended Use

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 the VITROS ECi/ECiQ Immunodiagnostic System.

Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection. The presence of anti-HBc may be used as an aid in the determination of exposure to HBV infection for individuals prior to HBV vaccination.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Summary and Explanation of the Assay

The VITROS Anti-HBc assay 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 bloods. Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection.

Principles of the Procedure

The VITROS Anti-HBc assay is performed using the VITROS Anti-HBc Reagent Pack and VITROS Immunodiagnostic Products Anti-HBc Calibrator on the VITROS ECi/ECiQ 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 increases the level and duration of the light produced. The light signals are read by the VITROS ECi/ECiQ Immunodiagnostic System. The amount of HRP conjugate bound is indicative of the concentration of anti-HBc present in the sample.

Assay Type Assay Time and Temperature

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Assay Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>Incubation time: 46 minutes</td>
</tr>
<tr>
<td></td>
<td>Time to first result: 55 minutes</td>
</tr>
<tr>
<td></td>
<td>Temperature: 37 °C</td>
</tr>
</tbody>
</table>

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Warnings and Precautions

For in vitro diagnostic use only.

Warning: Potentially Infectious Material

- Treat as if capable of transmitting infection.
- Handling of samples and assay components, their use, storage and solid and liquid waste disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation. (e.g. CLSI Guideline M29 s.s).

Human blood products provided as components of this pack, and of the VITROS Anti-HBc Calibrator, have been obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV) using FDA approved methods (enzyme immunoassays, EIA).

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent.

WARNING: Contains Kathon:
The assay reagent in the VITROS Anti-HBc Reagent Pack contains Kathon (0.5% w/w).
The conjugate reagent in the VITROS Anti-HBc Reagent Pack contains Kathon (0.5% w/w).
R36/38 – Irritating to eyes and skin.
R43 – May cause sensitization by skin contact.
R52/53 – Harmful to aquatic organisms, may cause long term adverse effects in the aquatic environment.
S24 – Avoid contact with skin.
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37/39 – Wear suitable gloves and eye/face protection.

Reagents

Reagent Pack Contents
One VITROS Anti-HBc Reagent Pack, 100 tests (CAT No. 680 1428) contains:
- 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 anti-microbial 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 anti-microbial agent (0.6% Kathon w/w)).

Reagent Pack Handling
- The reagent pack is supplied ready for use.
- Reagent packs do not need mixing.
- Avoid agitation, which may cause foaming or the formation of bubbles.
Reagent Pack Stability
When stored and handled as specified in the package labeling, the VITROS Anti-HBc Reagent Pack is suitable for use until the expiration date printed on the outside of the carton.

Reagent Pack Storage and Preparation
- Store the unopened reagent pack refrigerated at 2–8 °C (36–46 °F). Do not freeze.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Use opened reagent packs within 8 weeks.
- Store opened reagent packs in the VITROS ECi/ECiQ Immunodiagnostic System reagent supply, or refrigerated at 2–8 °C (36–46 °F) in a sealed reagent pack storage box that contains dry desiccant.

Specimen Collection and Preparation

Patient Preparation
No special patient preparation is necessary.

Recommended Specimen Types
Serum, EDTA or citrated plasma.

Specimens Not Recommended
Turbidity in samples may affect results.

Special Precautions
Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies. Because of the variety of sample collection devices available, it is not possible to issue a definitive statement on the performance of VITROS Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer’s instructions and is compatible with this assay.

Specimen Collection and Preparation
- Collect specimens using standard procedures.
- The VITROS Anti-HBc assay uses 50 μL of sample for each determination.
- For details on minimum fill volume of sample cups or containers, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator’s Guide.
- Mix samples, calibrator, and controls by inversion and bring to 15–30 °C (59–86 °F) before use.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Do not use heat inactivated samples.

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 board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator’s Guide for further information.
- 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 assay will not be completed within 8 hours, refrigerate samples at 2–8 °C (36–46 °F).
  - If the assay 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.

Assay Procedure
Materials Required But Not Provided

The following items are required to perform the VITROS Anti-HBc assay:
• VITROS ECi/ECiQ Immunodiagnostic System
• VITROS Anti-HBc Calibrator
• 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

Operating Instructions

Refer to the VITROS ECi /ECiQ Immunodiagnostic System Operator’s Guide for complete instructions on the operation of your VITROS ECi /ECiQ Immunodiagnostic System.

Sample Dilution

Rare patient samples occur that give high result ratios (s/c) greater than the normal negative population and which may be negative or positive for anti-HBc total antibody. These samples are defined by a result of greater than or equal to 4.8 s/c ratio and are most likely attributed to an unidentified interferent. For samples with results flagged “Equivocal”, dilute a measured aliquot with an appropriate volume of VITROS High Sample Diluent B to give a 1 in 20 dilution. For example, dilute 100 μL sample with 1.90 mL VITROS High Sample Diluent B and vortex mix. The VITROS Immunodiagnostic System does not need to be programmed for the dilution factor when testing the diluted sample using the VITROS Anti-HBc Reagent Pack. The results for a diluted sample should be interpreted as described in the VITROS Anti-HBc Reagent Pack Instructions for Use.

Calibration

Required Calibrator

VITROS Anti-HBc Calibrator

Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of the VITROS Anti-HBc Calibrator.

Calibration Procedure

• Calibration must be performed using a calibrator of the same lot number as the reagent pack.
• Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator’s Guide for detailed instructions on how to calibrate.

When to Calibrate

• Calibrate when the lot of reagent pack and calibrator changes.
• Calibrate every 28 days.

The VITROS Anti-HBc assay may also need to be recalibrated:
• After specified service procedures have been performed (see the VITROS ECi/ECiQ Immunodiagnostic System Operator’s Guide).
• If quality control results are consistently outside of the manufacturer’s or your acceptable range.

For additional information on when to calibrate, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator’s Guide.

Quality Control

Procedure Recommendations

• Choose control levels that check performance at clinically relevant points. 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.

- At least once every 24 hours.
- After specified service procedures or maintenance to critical parts or subsystems that might influence performance of the assay (see the VITROS ECI/ECiQ Immunodiagnostic System Operator’s Guide).
  - 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 acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
  - For more detailed information on quality control procedures, refer to the VITROS ECI/ECiQ Immunodiagnostic System Operator’s Guide.
  - 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.

Quality Control Material Selection
Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.
VITROS Anti-HBc Controls are recommended for use with the VITROS ECI/ECiQ Immunodiagnostic System. The performance of other commercial control fluids should be evaluated for compatibility with this assay before they are used for quality control. Appropriate quality control value ranges must be established for all commercially available quality control materials used with the VITROS Anti-HBc assay.

Quality Control Material Preparation and Storage
Refer to the manufacturer’s product literature for preparation, storage, and stability information.

Interpretation of Results and Expected Results
Results are calculated as a normalized signal, relative to the cutoff value (signal/cutoff, s/c). 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 ECI/ECiQ Immunodiagnostic System.

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

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 which requires dilution and re-assay.

<table>
<thead>
<tr>
<th>Result (s/c)</th>
<th>&lt;0.90</th>
<th>≥0.90 and ≤1.10</th>
<th>&gt;1.10 and &lt;4.80</th>
<th>≥4.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result Text</td>
<td>Reactive</td>
<td>Retest?</td>
<td>Negative</td>
<td>Equivocal</td>
</tr>
</tbody>
</table>

Final results should be manually interpreted using the algorithm below.
The magnitude of a VITROS Anti-HBc assay 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.

Expected Results

Approximately 65.7% (1111/1691) of the prospective subjects in Population I reported no recent or current signs or symptoms of hepatitis. Of these 1111 asymptomatic individuals, 25.3% were enrolled in Miami, FL, 36.5% were enrolled in Dallas, TX, and 38.2% were enrolled in Chicago, IL. The group was Caucasian (27.7%), African American (44.8%), Hispanic (18.7%), and Asian (4.8%) with the remaining 4% represented by other ethnic groups. The group was 51.6% male and 48.4% female and ranged in age from 5 to 89 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS Anti-HBc assay was reactive in 20.6% of the individuals in this group. The percent VITROS Anti-HBc reactive results observed in the asymptomatic population at each site was 4.5% at Miami, FL, 8.3% at Dallas, TX, and 7.8% at Chicago, IL.

The table below summarizes the distribution of VITROS Anti-HBc reactive and negative results among the study subjects without signs or symptoms of hepatitis, by age and gender.
Expected results for the VITROS Anti-HBc assay were also determined using unlinked samples from a population of pediatric and adolescent subjects in Utah at low risk for viral hepatitis (N=100). The group was 57% male and 43% female, and the subjects' ages ranged from two to 19 years. Three (3.0%) samples were reactive with the VITROS Anti-HBc assay and were tested with the reference anti-HBc assay. Two of these three were found to be reference anti-HBc assay reactive.

Limitations of the Procedure

- 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\textsuperscript{13}. 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.

Performance Characteristics

Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS Anti-HBc assay among individuals with signs or symptoms or biochemical manifestations (elevated liver function tests) of hepatitis and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, or known exposure events. Specimens were obtained from 1691 subjects prospectively enrolled at three geographically separated collection sites within the United States (Population I) located in Miami, FL (37.0%), Dallas, TX (28.1%) and Chicago, IL (34.9%). Specimens were also obtained from 315 subjects prospectively enrolled in an area in India with a high prevalence of viral hepatitis (Population II). Statistical testing performed to evaluate the homogeneity of the distribution of VITROS Anti-HBc s/c values across the four collection sites indicated that the data from Population I and Population II could not be pooled for statistical analysis.

The HBV disease classification for each subject was determined by a single point serological assessment using a hepatitis marker profile consisting of reference assays (previously licensed or approved by the FDA) for the detection of HBsAg, HBeAg, anti-HBc, anti-HBc IgM, anti-HBe, and anti-HBs (quantitative). The reference assays' procedures were adhered to during the clinical laboratory study.
The subjects in Population I were Caucasian (24.9%), African American (44.1%), Hispanic (22.4%) and Asian (3.7%), with the remaining 4.9% represented by other ethnic groups. The group was 52.4% male and 47.6% female, and ranged in age from 5 to 89 years. Testing of these specimens with the VITROS Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (37.0%), Port Jefferson, NY (34.9%) and Minneapolis MN (28.1%). Agreement of the VITROS Anti-HBc assay was assessed relative to the reference anti-HBc assay and HBV disease classification using serum samples from the 1691 subjects in Population I.

The subjects in Population II were Indian (100.0%). The group was 73.0% male and 27.0% female, and ranged in age from 18 to 90 years. Testing of these specimens with the VITROS Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (33.0%), Minneapolis MN (32.4%) and Los Angeles, CA (34.6%). Agreement of the VITROS Anti-HBc assay was assessed relative to the reference anti-HBc assay and HBV disease classification using serum samples from the 315 subjects in Population II.

**Results by Specimen Classification**

The data were analyzed following the assignment of HBV disease classifications based upon the positive (+)/negative (-) patterns for the six HBV serological reference markers. The table below summarizes how these classifications were derived. There were 28 unique reference marker profiles observed among the subjects in Populations I and II (24 unique patterns in Population I and 18 unique patterns in Population II) during the VITROS Anti-HBc clinical study.

<table>
<thead>
<tr>
<th>HBV Reference Marker Profiles and HBV Disease Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference HbsAg</td>
</tr>
<tr>
<td>Reference HBsAb</td>
</tr>
<tr>
<td>Reference HbeAg</td>
</tr>
<tr>
<td>Reference Total HBc</td>
</tr>
<tr>
<td>Reference Anti-HBc 3 TI mIU/mL</td>
</tr>
<tr>
<td>HBV Disease Classification</td>
</tr>
<tr>
<td>Acute</td>
</tr>
<tr>
<td>Chronic</td>
</tr>
<tr>
<td>Early Recovery</td>
</tr>
<tr>
<td>Recovery</td>
</tr>
<tr>
<td>Recovred</td>
</tr>
<tr>
<td>Not Previously Infected with HIV</td>
</tr>
<tr>
<td>Underinterpretable</td>
</tr>
<tr>
<td>Interpretale</td>
</tr>
</tbody>
</table>

Positive = Reference HbsAg assay reactive and confirmed by neutralization.
Negative = Reference HbsAg assay negative or not confirmed by neutralization.

**Comparison of Results**

The table below compares the VITROS Anti-HBc results with the reference anti-HBc results by specimen classification for the subjects in Population I.
Percent Agreement
Positive and negative percent agreement between the VITROS Anti-HBc assay and the reference anti-HBc assay were calculated for subjects in Population I (N=1691) with various HBV disease classifications, and for the overall study population. The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals.

The positive percent agreement with the reference anti-HBc assay was determined by dividing the number of reactive VITROS Anti-HBc results by the total number of subjects reactive with the reference anti-HBc assay. As a result of this study, the overall positive percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population I was estimated to be 92.38% (400/433, with a 95% exact confidence interval of 89.46% to 94.70%).

The negative percent agreement with the reference anti-HBc assay was determined by dividing the number of negative VITROS Anti-HBc results by the total number of subjects negative with the reference anti-HBc assay. As a result of this study, the overall negative percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population I was estimated to be 99.60% (1253/1258, with a 95% exact confidence interval of 99.07% to 99.87%).

Positive and negative percent agreement between the VITROS Anti-HBc assay and the reference anti-HBc assay were also calculated for subjects in Population II (N=315) with various HBV disease classifications, and for the overall study population. The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals.
The positive percent agreement with the reference anti-HBc assay was determined by dividing the number of reactive VITROS Anti-HBc results by the total number of subjects repeatedly reactive with the reference anti-HBc assay. As a result of this study, the overall positive percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 98.56% (273/277, with a 95% exact confidence interval of 96.34% to 99.61%).

The negative percent agreement with the reference anti-HBc assay was determined by dividing the number of negative VITROS Anti-HBc results by the total number of subjects negative with the reference anti-HBc assay. As a result of this study, the overall negative percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 100.0% (38/38, with a 95% exact confidence interval of 90.75% to 100.0%).

The performance of the VITROS Anti-HBc assay was further evaluated among archived serum samples from subjects with clinical and laboratory documentation of acute or chronic (HBsAg present for ≥6 months) HBV infection. The table below summarizes the performance of the VITROS Anti-HBc assay in samples from subjects with documented acute or chronic HBV infection.

Clinical Performance of the VITROS Anti-HBc Assay in Pre-Vaccination Samples
Serum samples obtained from 41 individuals immediately prior to HBV vaccination were tested with the VITROS and reference anti-HBc assays. The results are shown below for both assays.

Seroconversion Panels
Six commercially available seroconversion panels were tested. The VITROS and reference anti-HBc assay results are summarized below. The table lists the first bleed of each panel that tested reactive with the VITROS and the reference assays as well as the difference between the two assays in identifying the first reactive panel member by number of days.

Potentially Cross-Reacting Subgroups
Samples with evidence of hepatitis A virus infection (HAV) or hepatitis C virus infection (HCV) were identified in a population of 1691 samples prospectively collected from subjects in the U.S with signs or symptoms of, or at risk for, viral hepatitis.
(Population I). The tables below compare VITROS Anti-HBc results with reference anti-HBc results according to the HBV disease classifications assigned to the study subjects.

<table>
<thead>
<tr>
<th>HBV Disease Classification</th>
<th>Reference Anti-HBc Result</th>
<th>VITROS Anti-HBc Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>Negative</td>
</tr>
<tr>
<td>Acute</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early Recovery</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recovery</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recovered</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>HBV vaccine response</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not Previously infected</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uninterpretable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Samples with evidence of hepatitis A virus infection (HAV) and/or hepatitis C virus infection (HCV) were identified in a population of 315 samples prospectively collected from subjects in an area in India with a high prevalence of viral hepatitis (Population II). The tables below compare VITROS Anti-HBc results with reference anti-HBc results according to the HBV disease classifications assigned to the study subjects.

<table>
<thead>
<tr>
<th>HBV Disease Classification</th>
<th>Reference Anti-HBc Result</th>
<th>VITROS Anti-HBc Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>Negative</td>
</tr>
<tr>
<td>Acute</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early Recovery</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Recovery</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>Recovered</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>HBV vaccine response</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not Previously infected</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uninterpretable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>140</td>
<td>1</td>
</tr>
</tbody>
</table>

The specificity of the VITROS Anti-HBc assay was evaluated by testing 232 samples from 16 potentially cross-reacting subgroups. Patient samples from the following sub-groups were tested: HAV, HEV, HCV, non-viral liver disease, autoimmune disease (rheumatoid arthritis and systemic lupus erythematosis), CMV, EBV, HSV, parvovirus B19 infection, rubella, syphilis, toxoplasmosis, HIV 1/2 antibody positive, HTLV 1/2 antibody positive, and HBV vaccine recipients.

Of the 232 samples tested, 230 were observed to be negative. One autoimmune disease (rheumatoid arthritis) sample was
initially reactive in the VITROS Anti-HBc assay, but was negative on repeat determination. One Syphilis sample was reactive initially in the VITROS Anti-HBc assay and also on repeat determination.

A total of 20 cord blood patient samples were tested in the VITROS Anti-HBc assay. In testing the cord blood samples, 1 out of 20 samples was found to give a repeatedly reactive result in the VITROS Anti-HBc assay. This repeatedly reactive sample was also repeatedly reactive in the reference method.

Substances that do not Interfere
The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 10 blood donors. The results (mean of test results at each level of interferent) demonstrate that hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL) and triolein (up to 3000 mg/dL), cause no misclassification of results. Anti-HBc spiked samples were tested near the cut-off (cut-off s/c=1.00), and were observed to remain reactive at all levels tested with each potential interferent. Similarly no interference was observed in samples not spiked with anti-HBc (Negative), with anti-HBc values remaining above 2.00 s/c.

Precision
Precision was evaluated on a different VITROS ECi/ECiQ Immuno diagnostic System at three external sites, using one reagent pack and calibrator kit lot. At least two replicates each of a three member panel were assayed on a single occasion per day on 20 different days. The data shown in the table were rounded following all calculations.
Precision was further evaluated incorporating between site and between lot variation. The study was performed at three external sites using three reagent lots. At least three replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates (CV (%)) were derived from a variance component analysis. The data shown in the table were rounded following all calculations.

### References
