Laboratory Procedure Manual

Analyte: Hepatitis B Surface Antigen (HBsAg)

Matrix: Serum

Method: HBsAg
VITROS Immunodiagnostic Products (REF 680 1322)

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As performed by: Assay Development and Diagnostic Reference Laboratory (ADDRL)
Laboratory Branch
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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>LBDHBG</td>
<td>Hepatitis B surface antigen</td>
</tr>
</tbody>
</table>
INSTRUCTIONS FOR USE HBsAg
VITROS Immunodiagnostic Products

HBsAg Reagent Pack
REF 680 1322

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 hepatitis B surface antigen (HBsAg) in human serum and plasma (heparin, EDTA, and sodium citrate) 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. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates who are at high risk of acquiring HBV during the perinatal period.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors. Assay performance characteristics have not been established when the VITROS HBsAg assay is used in conjunction with other manufacturers’ assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Summary and Explanation of the Assay
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. 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.

Detection of HBsAg by sensitive enzyme immunoassays was described by Engvall and Perlmann, Engvall, Jonsson and Perlmann, and VanWeemen and Schuurs in 1971. Subsequently, solid-phase sandwich enzyme immunoassays for the detection of HBsAg were described by Wisdom, Wolters et al, and Wei et al. Production, characterization and application of monoclonal antibodies for the detection of HBsAg have also been described.

Principles of the Procedure
The VITROS HBsAg assay is performed using the VITROS HBsAg Reagent Pack and VITROS Immunodiagnostic Products HBsAg Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System (VITROS Immunodiagnostic System).

An immunometric technique is used. This involves the simultaneous reaction of HBsAg in the sample with mouse monoclonal anti-HBs antibody coated onto the wells and a horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBs antibody in the conjugate. Unbound conjugate is removed by washing.

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 Immunodiagnostic System. The amount of HRP conjugate bound is indicative of the level of HBsAg present in the sample.

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Assay Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunometric assay</td>
<td>Incubation time 29 minutes</td>
</tr>
<tr>
<td></td>
<td>Time to first result 37 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**

*Human blood products provided as components of this pack have been obtained from donors who were tested individually and found to be negative for HBsAg, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays, EIA). The VITROS HBsAg Calibrator contains human HBsAg purified from donors who were tested individually and found to be negative for antibodies to HIV 1+2 and HCV (using EIA). The purified HBsAg has been heat inactivated (10 hours at 60 °C). Treat as if capable of transmitting infection. 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. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation. 14, 15*

**WARNING: Contains Kathon**

*The reagents contain Kathon. 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. S37: Wear suitable gloves.*

**Reagents**

**Reagent Pack Contents**

One VITROS HBsAg Reagent Pack; 100 tests (CAT No. 680 1322) contains:
- 100 coated wells (mouse monoclonal anti-HBs (directed to the “a” region determinant), coated at 1 μg/well)
- 6.2 mL conjugate reagent (HRP- mouse monoclonal anti-HBs, 0.9 μg/mL) in buffer with bovine serum albumin, goat serum, and antimicrobial agent (Kathon 1% w/v)
- 8.4 mL assay reagent with human serum, newborn calf serum, mouse serum, and antimicrobial agent (Kathon 1% w/v)

**Reagent Pack Handling**

- The reagent pack is supplied ready for use.
- 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 HBsAg Reagent Pack is suitable for use until the
expiration date printed on the outside of the cart.

**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 Immunodiagnostic System reagent supply, or refrigerated at 2–8 °C (36-46 °F) in a sealed reagent pack storage box that contains dry desiccant.
- Exposure of Reagent Pack and Calibrator to temperatures>30 °C (86 °F) for extended periods of time may affect assay performance.

**Specimen Collection and Preparation**

**Patient Preparation**

No special patient preparation is necessary.

**Recommended Specimen Types**

Serum, EDTA, heparin, or citrated plasma. Heparin and citrate have been shown to lower the signal/cutoff (s/c) values in some HBsAg reactive samples. High negative results (0.80–0.99 s/c) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required. Follow manufacturer's instructions for using plasma collection containers with anticoagulants.

**Specimens Not Recommended**

Turbidity in samples may affect assay 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 HBsAg assay uses 80 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, calibrators, 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 a falsely elevated result.

**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 VITROS Immunodiagnostic 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 blood 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 the sample at 2–8 °C (36–46 °F). If the assay will not be completed within 48 hours, or for shipment of samples, freeze 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 HBsAg assay:

- VITROS Immunodiagnostic System
- VITROS HBsAg Calibrator
- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials, such as VITROS Immunodiagnostic Products HBsAg Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

**Operating Instructions**

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

**Calibration**

**Required Calibrators**

VITROS HBsAg Calibrator

**Calibrator Preparation, Handling, and Storage**

Refer to the calibrator instructions for use for information on the use of VITROS HBsAg 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 HBsAg assay may also need to be calibrated:

- 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 HBsAg decision point (signal/cutoff ≥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. 19
- 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. 20
VITROS HBsAg Controls are recommended for use with the VITROS 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 should be established for all commercially available quality control materials used with the VITROS HBsAg 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 Immunodiagnostic System.

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

Patient sample results will be displayed with a "Negative", "Retest?", or "Positive" label. An initial result labeled with "Retest?" indicates a sample that requires repeat testing for HBsAg.

\[
\begin{array}{ccc}
\text{Result (s/c)} & < 0.90 & \geq 0.90 \text{ and } \leq 5.00 & > 5.00 \\
\text{Result Text} & \text{Negative} & \text{Retest?} & \text{Positive} \\
\end{array}
\]

Final results should be manually interpreted using the algorithm below.

Testing Algorithm
Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS HBsAg assay upon completion of all testing steps required in the testing algorithm.

<table>
<thead>
<tr>
<th>Final VITROS HBsAg Assay Result (s/c)</th>
<th>Conclusion from Testing Algorithm</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.00 and ≤ 5.00</td>
<td>Reactive</td>
<td>Sample is positive for HBsAg.</td>
</tr>
<tr>
<td>≥ 1.00 and ≤ 5.00</td>
<td>Reactive</td>
<td>Sample is reactive for HBsAg. If a reactive result is confirmed by supplemental tests such as the VITROS Immunodiagnostics Products HBsAg Confirmatory Kit, the specimen is positive for HBsAg.</td>
</tr>
<tr>
<td>&gt; 5.00</td>
<td>Positive</td>
<td>Specimen is positive for HBsAg.</td>
</tr>
</tbody>
</table>

- The magnitude of a VITROS HBsAg assay result cannot be correlated to an endpoint titer.
- The ability of the VITROS HBsAg assay to detect HBV mutants has not been determined. Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.
- Heparin and citrate have been shown to lower the signal/cutoff (s/c) values in some HBsAg reactive samples. High negative results (0.80–0.99 s/c) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required.

Expected Results

Approximately 66.1% (1439/2177) of the prospective subjects participating in the VITROS HBsAg clinical study were
asymptomatic and reported no recent or current signs or symptoms of hepatitis. Of these individuals, 20.9% were enrolled in Miami, FL, 46.1% were enrolled in Dallas, TX, 32.6% were enrolled in Chicago, IL, and 0.4% were enrolled in New York, NY. The group was Caucasian (28%), African American (46%) Hispanic (18%), and Asian (4%) with the remaining 4% represented by three or more ethnic groups. The group was 54% male and 46% female and ranged in age from 5 to 96 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS HBsAg assay was positive in 3.3% of the individuals in this group. The percent VITROS HBsAg positive results observed in the asymptomatic population at each site was 4.3% at Miami, FL, 3.5% at Dallas, TX, 2.0% at Chicago, IL, and 20% at New York, NY.

The table below summarizes the percent VITROS HBsAg positive and negative results by gender and age range.

### Limitations of the Procedure

- Heterophilic, e.g. human anti-mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products.
- Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.
- HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with hepatitis B virus. Levels of HBsAg may be undetectable both in early infection and late after infection. In rare cases HBsAg tests do not detect certain HBV mutant strains.
- The analytical sensitivity of the VITROS HBsAg assay was determined to be 0.085 IU/mL World Health Organization (WHO) 1st International Reference Standard 80/549), 0.030 PEI Units/mL (commercial ad subtype sensitivity panel), and 0.019 PEI Units/mL (commercial ay subtype sensitivity panel).
- Assay performance characteristics have not been established for any other specimen matrices than serum or heparin, EDTA, and sodium citrate anticoagulated plasma.
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture.
- It has been shown that up to 498 μg HBsAg/mL does not create a high dose hook effect that will interfere with this assay.

### Performance Characteristics

#### Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS HBsAg assay with individuals with signs or symptoms of hepatitis. Also included were individuals at high risk of HBV infection due to lifestyle, behavior, occupation, or known exposure events. Specimens were obtained from collection sites located in Miami, FL (32%), Dallas, TX (36%), Chicago, IL (30%), and New York, NY (2%). The group was Caucasian (27%), African American (44%), and Hispanic (22%), with the remaining 7% represented by other ethnic groups. The group was 54% male and 46% female and ranged in age from five to 96 years. 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). All reference assays used were from a single manufacturer. The reference assays' procedures were adhered to during the clinical laboratory study. Testing of these specimens occurred at hospital...
associated diagnostic laboratories located in Miami, FL (32%), Dallas, TX (36%), and Port Jefferson, NY (32%). Agreement of the VITROS HBsAg assay was assessed relative to the reference HBsAg confirmed results and the specimen classification using serum samples from 2156 of the 2177 subjects enrolled.*

**Results by Specimen Classification**

The data were analyzed following the assignment of specimen classification based upon the positive (+) / negative (-) patterns for the six HBV serological reference markers. The table below summarizes how these classifications were derived. There were 24 unique reference marker patterns observed in the VITROS HBsAg clinical study.

* HBV disease classification could not be determined for 21 of the 2177 subjects due to incomplete reference marker profiles (missing one or more results for the panel of six HBV reference markers). These 21 subjects were excluded from the analysis.

**Comparison of Results**

The table below compares the VITROS HBsAg results with the reference HBsAg results by specimen classification for the prospective sample population.

The results are broken out further where initial VITROS HBsAg results required repeat testing and confirmation (s/c ≥1.00 and ≤ 5.00), and positive samples where no further VITROS testing was required (s/c >5.00).
Percent Agreement

The table below summarizes the percent agreement between the VITROS HBsAg assay and the reference HBsAg assay for each specimen classification for the prospective sample population. The table provides the 95% exact confidence interval.

Percent Agreement of the VITROS HBsAg Assay for Subjects with Clinically Diagnosed Acute or Chronic HBV Infection

The performance of the VITROS HBsAg assay was further evaluated among archived serum samples from subjects based on documented clinical status or diagnosis of acute (demonstrated seroconversion or HBV reference marker profile) or chronic (HBsAg present for ≥ 6 months) HBV infection. Samples were obtained prospectively and from commercial and site archives. The table below summarizes the percent agreement of the VITROS HBsAg assay in samples from subjects with documented acute or chronic HBV infection.

Seroconversion Panels

Seventeen HBV seroconversion panels were obtained from two commercial vendors (6280–6293 and PHM920–PHM933). These panels were obtained from donors in the early stages of seroconversion from HBsAg negative to HBsAg positive status and contained individual samples in which HBsAg was the only detectable HBV marker, as determined by historical HBV
marker data provided by the manufacturers.
The table below presents a summary of the results of testing of the 17 panels with the VITROS and reference HBsAg assays.

<table>
<thead>
<tr>
<th>Panel ID</th>
<th>Days to HBsAg Reactive Result from Initial Draw Date</th>
<th>Difference in Days to HBsAg Reactive Result (Reference — VITROS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>62B</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>62B</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>62B</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>62B</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>62B</td>
<td>46</td>
<td>4</td>
</tr>
<tr>
<td>62B</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>62B</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>62B</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>62B</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>62B</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>62B</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>62B</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>62F</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>PHM20</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Primaq</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PM22E2</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>PHM23</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Clinical Performance in Pregnant Women
Prospectively collected and archived serum samples from healthy, pregnant women at low risk or high risk for exposure to HBV were tested to assess the clinical performance of the VITROS HBsAg assay in screening for hepatitis B infection to identify neonates at high risk of acquiring HBV during the perinatal period. A total of 545 samples were prospectively collected during the clinical study in several different locations in the US. An additional 199 frozen archived samples were obtained from a commercial vendor. These frozen archived samples had been prospectively collected from women at low risk for viral hepatitis in several different locations in the US. Of the combined 744 prospectively collected and archived samples, 52% were obtained in Florida, 24% were obtained in Texas, 23% were obtained in California and 1% were obtained in Connecticut. Of the combined population, 35.9% were obtained during the first trimester, 34.1% during the second trimester and 30.0% during the third trimester. The following table furnishes a breakdown of the study population.

<table>
<thead>
<tr>
<th>Demographics of Pregnant Women at Low or High Risk for Viral Hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>TRIMESTER</td>
</tr>
<tr>
<td>First</td>
</tr>
<tr>
<td>Second</td>
</tr>
<tr>
<td>Third</td>
</tr>
<tr>
<td>ETHNICITY</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td>African American</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>Indian</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>AGE (Years)</td>
</tr>
<tr>
<td>11-35</td>
</tr>
<tr>
<td>36-45</td>
</tr>
</tbody>
</table>

Agreement of the VITROS HBsAg assay was assessed relative to the reference HBsAg results using serum samples obtained from a total of 744 women at low risk or high risk for HBV infection. The tables below compare the VITROS and reference HBsAg assays among the overall population of pregnant women by risk and trimester.
Overall VITROS and Reference HBsAg Results Among Pregnant Women

Frequency of Reactivity of the VITROS HBsAg Assay in Pregnant Women

The table below summarizes the frequency of reactivity of the VITROS HBsAg assay from a total of 744 women at low risk and high risk for HBV infection.

<table>
<thead>
<tr>
<th>VITROS HBsAg Result</th>
<th>Reference HBsAg Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Positive and Negative Percent Agreement of the VITROS and Reference HBsAg Assays in Pregnant Women

The table below summarizes the percent agreement between the VITROS HBsAg assay and the reference HBsAg assay for this population. The table provides the 95% exact confidence intervals.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Positive Percent Agreement</th>
<th>95% Exact Confidence Interval</th>
<th>Negative Percent Agreement</th>
<th>95% Exact Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant Women</td>
<td>100 (95)</td>
<td>47.4 to 100.0</td>
<td>100.0 (73.7 to 136.3)</td>
<td></td>
</tr>
</tbody>
</table>

Performance of Neonate Serum

In order to determine if neonate serum may be tested with the VITROS HBsAg assay, cord blood was used as a surrogate for neonate serum. A total of 60 individual cord blood and ten individual serum samples were tested in the VITROS HBsAg assay. In testing the cord blood samples, 0 out of 60 were found to give a reactive result. None of the serum samples were initially reactive. Upon spiking the cord blood samples with HBsAg to a target of 2.0 s/c, 58 out of 60 samples gave a reactive result. Two samples were found to contain a significant amount of anti-HBs. These two specimens were not evaluated due to the HBsAg result being below the reactive level. To remove systematic bias, each serum sample mean was evaluated to each cord blood mean. The table below shows the amount of bias for the cord blood samples from serum.

<table>
<thead>
<tr>
<th>Negative and Positive Percent (%) HBsAg Cord Blood Bias Related to Serum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5% s/c</td>
<td>-2%</td>
<td>2%</td>
</tr>
<tr>
<td>5% to 25%</td>
<td>-1%</td>
<td>3%</td>
</tr>
<tr>
<td>25% to 75%</td>
<td>1%</td>
<td>4%</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>2%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Potentially Cross-Reacting Subgroups

The specificity of the VITROS HBsAg assay was evaluated by testing 249 samples from 16 potentially cross-reacting subgroups. All of the samples were previously classified as HBsAg negative in other commercially available assays. Samples found to be ≥ 1.00 by the VITROS HBsAg assay were retested in duplicate. A summary of the results is given in the table below.
Substances that do not Interfere

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

Analytical Sensitivity

Detection of a known level of HBsAg is a function of the assay’s analytical sensitivity (that is, the dependency of the assay result on the HBsAg level) as well as the assay precision. To examine the analytical sensitivity of the VITROS HBsAg assay, three standard series with known levels were evaluated. Duplicate determinations of each panel member were obtained using three lots of reagents. The HBsAg level at the assay’s cutoff was estimated from a linear regression analysis.

As a demonstration of performance of subtype detection, the VITROS HBsAg assay tested with the French ADM SFTS 1996 Sensitivity panel is presented below. The panel contains 20 individual samples representing 10 subtypes with a known, predetermined HBsAg concentration. Single determinations of the panel members with the VITROS HBsAg assay were made. The VITROS HBsAg assay demonstrated detection of all subtypes in the French ADM SFTS 1996 Sensitivity panel. Ten panel members consisted of the more common ad/ay subtype. Nine panel members represented the less commonly encountered subtypes and are depicted in the graph below.
**Precision**

Precision was evaluated on a different VITROS Immunodiagnostic System at three external sites using one lot of reagent. With one exception, at least two replicates each of a three member panel were assayed on a single occasion per day on up to 20 different days. The data shown in the table were rounded following all calculations.

![Precision Table](image)

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 five 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.

![Between Site and Lot Table](image)

**References**


Glossary of Symbols

The following symbols may have been used in the labeling of this product.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do Not Return</td>
<td></td>
</tr>
<tr>
<td>Use by or Expiration Date (Year-Month-Day)</td>
<td></td>
</tr>
<tr>
<td>Lot Number</td>
<td></td>
</tr>
<tr>
<td>Serial Number</td>
<td></td>
</tr>
<tr>
<td>GCN</td>
<td>Catalog Number or Product Code</td>
</tr>
<tr>
<td>ATTENTION</td>
<td>Attention: See Instructions for Use</td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>In vitro Diagnostic Medical Device</td>
<td></td>
</tr>
<tr>
<td>Authorized Representative in the European Community</td>
<td></td>
</tr>
<tr>
<td>Contains Sufficient for &quot;X&quot; Tests</td>
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</tr>
<tr>
<td>Upper Limit of Temperature</td>
<td></td>
</tr>
<tr>
<td>Lower Limit of Temperature</td>
<td></td>
</tr>
<tr>
<td>Temperature Limitation</td>
<td></td>
</tr>
<tr>
<td>Consult Instructions for Use, &quot;X&quot; Version</td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td></td>
</tr>
<tr>
<td>Hemat</td>
<td></td>
</tr>
<tr>
<td>Corrosive</td>
<td></td>
</tr>
<tr>
<td>Flammable</td>
<td></td>
</tr>
<tr>
<td>Fragile, Handle with Care</td>
<td></td>
</tr>
<tr>
<td>Keep Dry</td>
<td></td>
</tr>
<tr>
<td>This end up</td>
<td></td>
</tr>
<tr>
<td>Der Gute Punkt (the Green Dot); Manufacturer fulfills center-posting material under disposal management regulations</td>
<td></td>
</tr>
</tbody>
</table>

Revision History

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