

## SUMMARY OF SAFETY AND EFFECTIVENESS

### 1. General Information

#### 1.1. Name and Address of Applicant

Ortho-Clinical Diagnostics, Inc  
100 Indigo Creek Drive  
Rochester NY 14626-5101

#### 1.2. Device Trade Name

*Vitros* Immunodiagnostic Products HBsAg Reagent Pack  
*Vitros* Immunodiagnostic Products HBsAg Calibrator  
*Vitros* Immunodiagnostic Products HBsAg Confirmatory Kit

#### 1.3. Classification (Generic) Name of Device

Hepatitis B Surface Antigen (HBsAg) Assay  
Hepatitis B Surface Antigen Assay Calibrator  
Hepatitis B Surface Antigen (HBsAg) Confirmatory Kit

#### 1.4. PMA Number:

P000044

#### 1.5. Date of Panel Recommendation:

Pursuant to Section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this Panel.

#### 1.6. Date of Notice of Approval To Applicant:

APR 27 2001

### 2. Indications for Use

#### 2.1. *Vitros* HBsAg Assay:

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 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.

## 2.2. Vitros Immunodiagnostic Products HBsAg Calibrator:

For use in the calibration of the *Vitros* Immunodiagnostic System for the qualitative *in vitro* determination of hepatitis B surface antigen (HBsAg) in human serum or plasma (heparin, EDTA, and sodium citrate) using *Vitros* HBsAg Reagent Packs. The *Vitros* HBsAg Calibrator has been validated for use only on the *Vitros* System with the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack. Refer to the *Vitros* HBsAg Reagent Pack instructions for use for further details.

## 2.3. Vitros HBsAg Confirmatory Kit:

For the qualitative confirmation of hepatitis B surface antigen (HBsAg) in human serum and plasma (heparin, EDTA, and sodium citrate) specimens that have been found to be repeatedly reactive using the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack and the *Vitros* Immunodiagnostic Products HBsAg Calibrator with the *Vitros* ECi Immunodiagnostic System.

# 3. Device Description

## 3.1. Principle of Device Methodology

3.1.1. The *Vitros* ECi Immunodiagnostic System (*Vitros* Analyzer) allows for the determination of analytes in human samples (for example, serum and plasma). All assays on the analyzer employ an enhanced chemiluminescence detection reaction. The *Vitros* Analyzer is fully automated with a refrigerated on board assay monitoring system. The analyzer supports all standard bar code symbologies and has a throughput of up to 90 assays per hour. The analyzer also provides menu driven software, which can be accessed, from a high-resolution touch screen monitor.

3.1.2. The *Vitros* HBsAg assay incorporates an immunometric technique involving the simultaneous reaction of HBsAg present in the specimen with mouse monoclonal anti-HBs coated on the wells and a second HRP-labeled mouse monoclonal anti-HBs antibody in the conjugate. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by luminescence. 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 *Vitros* Analyzer. The amount of HRP conjugate bound is indicative of the concentration of HBsAg present.

3.1.3. The *Vitros* HBsAg Calibrator allows calibration of the *Vitros* HBsAg assay. Calibration is lot specific. A Master Calibration is established for each new

reagent lot by performing multiple assays. The lot-specific parameter, the expected calibrator signal and the data that enables the *Vitros* System to calculate the cut-off value are encoded on the lot calibration card. Scanning the lot calibration card loads the encoded data into the *Vitros* System. When the calibrator is processed, the validity of the calibration is assessed against a quality parameter that compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cut-off value is calculated and stored for use with any reagent pack of that lot. Recalibration is required after a predetermined calibration interval, or when a different reagent pack lot is loaded.

- 3.1.4. The *Vitros* HBsAg Confirmatory Kit uses the principle of specific antibody neutralization to confirm the presence of HBsAg. Aliquots of samples that are reactive are added to two sample containers: one aliquot is incubated with a reagent containing the neutralizing antibody (human anti-HBsAg); the second aliquot is incubated with the sample diluent, a non-neutralizing control reagent. The confirmatory antibody binds to HBsAg in the specimen and inhibits its reaction in the *Vitros* HBsAg assay. This results in a reduced signal to cut off result compared to the result of the non-neutralized control specimen.

### 3.2. Kit Configuration and Components

- 3.2.1. The *Vitros* HBsAg Reagent Pack is composed of 3 reagents:

- Conjugate reagent [horseradish peroxidase labeled mouse monoclonal anti-HBs (directed to the “a” region determinant) with goat serum and bovine serum albumin in buffer with antimicrobial agent (Kathon)]
- Assay reagent [with human serum, newborn calf serum, mouse serum and antimicrobial agent (Kathon)]
- Coated microwells [mouse monoclonal anti-HBs (coated at 1 µg/mL per well)]

- 3.2.2. The *Vitros* HBsAg Confirmatory Kit contains:

- Confirmatory Antibody reagent (anti-HBs positive human serum) each binds  $\geq 1$  µg HBsAg, with antimicrobial agent. (1% Kathon w/v.)
- Sample Diluent (human serum, non-reactive for HBsAg, anti-HBs negative), with antimicrobial agent (0.5% bronidox w/v, 27 mL).

- 3.2.3. The *Vitros* HBsAg Calibrator contains:

- Inactivated HBsAg *ad* subtype in buffer with bovine serum albumin and antimicrobial agent (Kathon). The Calibrator is supplied ready for use.

- 3.2.4. In addition, the following components are required

- *Vitros* ECi Immunodiagnostic System (*Vitros* Analyzer) – dedicated instrumentation that provides automated analysis of the *Vitros* assays. *Vitros*

Immunodiagnostic Products Signal Reagent and *Vitros* Immunodiagnostic Products Universal Wash Reagent are Universal Reagents used in all *Vitros* System assays.

**4. Contraindications**

None known

**5. Warnings and Precautions**

Warnings and precautions for users of the *Vitros* HBsAg Reagent Pack and Calibrator and the *Vitros* HBsAg Confirmatory Kit are stated in the respective product labeling.

**6. Alternate Practices and Procedures**

Determining the presence of HBsAg in individuals may be achieved by using a variety of commercially available, FDA licensed serological tests. Additionally, when test results are used in combination with a physician's assessment and supplemental clinical laboratory serological and biochemical testing, infection with HBV can be identified.

**7. Marketing History**

Countries where the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack and Calibrator and the *Vitros* HBsAg Confirmatory Kit have been or can be distributed are presented in the following table.

Country	
Argentina	Netherlands
Australia	Norway
Belgium	Panama
Canada	Philippines
Chile	Poland
China	Portugal
Columbia	Romania
Czech Republic	Russia
Denmark	Saudi Arabia
France	Singapore
Germany	Slovak Republic
Iceland	Slovenia
India	Spain
Indonesia	Sweden
Italy	Switzerland
Japan	Taiwan
Korea	Thailand
Malaysia	Turkey
Mexico	UK

This product has not been withdrawn from any of these markets for any reason.

## 8. Potential Adverse Effects of the Device on Health

Since the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack, *Vitros* Immunodiagnostic Products HBsAg Calibrator, and *Vitros* Immunodiagnostic Products HBsAg Confirmatory Kit are for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient.

However, failure of the device to perform as indicated or human error in use of the device may lead to a false result. A false positive result using an HBsAg assay is not considered a patient or public health concern as a positive result in a clinical lab setting is usually followed up with supplemental testing. Either additional HBV marker testing is performed or an HBsAg positive result is confirmed by neutralization. An exception to this is using HBsAg tests to screen pregnant women for the presence of HBsAg. This testing helps to determine if a neonate is at high risk of acquiring HBV during the prenatal period. Pregnant women are tested during an early prenatal visit. If they are HBsAg nonreactive during this testing, and at high risk for HBV infection, they are re-tested during the third trimester. If the result is positive, it is recommended that hepatitis B immune globulin (HBIG) and vaccine be provided to the newborn within 12 hours of birth. If an assay is false positive and the newborn receives HBIG, the newborn would be exposed to the risks of receiving a human source product.

The risks of a false negative result in a diagnostic setting are highest when testing pregnant women because HBsAg may be the only marker used. If the result is negative then the child is vaccinated within 2 months of birth. If the result is incorrect (false negative), the neonate is at a higher risk of acute and chronic HBV infection, since HBIG and vaccine would not be provided within 12 hrs of birth.

In addition, from time to time false negative results due to gene mutation have been reported for the HBsAg assays produced by a number of different manufacturers. Because of the complexity of the mutations that can occur, no manufacturer can guarantee to detect all infectious donors and patients.

A false positive result using an HBsAg confirmatory neutralization procedure is not considered a patient or public health concern because in order for a false positive to occur, the control sample (non-neutralized result) and the percent neutralization (in the neutralized tube) would both have to be incorrect for a reported false positive result. If this situation were to occur, the implications would be the same as described for false positive results for HBsAg assays

A false negative result using an HBsAg confirmatory neutralization procedure could occur if the neutralized sample were incorrect either due to a falsely increased signal with the neutralized sample, or due to some other malfunction, laboratory or technician error when assayed. A falsely increased signal could be interpreted as a failure to neutralize. If this

situation were to occur, the implications would be the same as described for false negative results for HBsAg.

## 9. Summary of Non Clinical Studies

### 9.1. Instrumentation

Software and hardware verification testing was performed for the *Vitros* ECI Immunodiagnostic System (*Vitros* Analyzer). Appropriate information and study results were furnished demonstrating that the *Vitros* Analyzer hardware and software, used with the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack, functioned as described and had appropriate safeguards in place.

### 9.2. Analytical Sensitivity

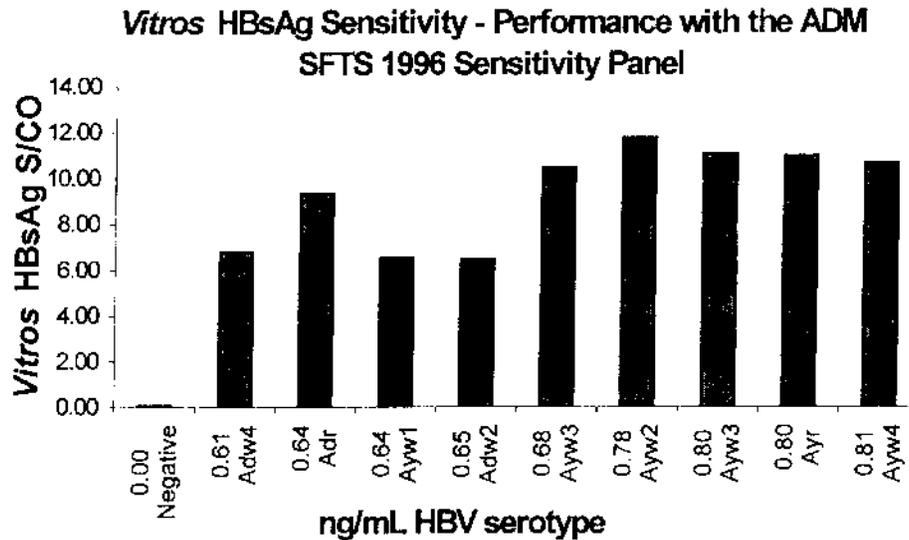
To examine the sensitivity of the *Vitros* HBsAg assay three HBsAg panels with known concentration were evaluated: the World Health Organization (WHO) 1<sup>st</sup> International Reference Standard, the Boston Biomedica, Inc. (BBI) HBsAg Sensitivity Panel (*ad* subtype) and the BBI HBsAg Sensitivity Panel (*ay* subtype).

For the WHO 1<sup>st</sup> International Reference Standard HBsAg concentrations greater than or equal to 0.125 IU/ml were detected 100% of the time. HBsAg concentrations of less than or equal to 0.075 IU/mL were detected 0% of the time. The HBsAg concentration at cut-off ( $s/c$  (signal/cutoff) = 1.00) estimated from the linear regression analysis was 0.085 IU/mL (95% CI = 0.051 to 0.118 IU/mL).

For the BBI HBsAg Sensitivity panel (*ad* subtype), HBsAg concentrations greater than or equal to 0.04 PEI (Paul Ehrlich Institute) Units/ml were detected 100% of the time. HBsAg concentrations of less than or equal to 0.01 PEI Units/mL were detected 0% of time. The HBsAg concentration at cut-off ( $s/c=1.00$ ), estimated from the linear regression analysis was 0.030 PEI Units/mL (95% CI = 0.007 to 0.054 PEI Units/mL).

For the BBI HBsAg Sensitivity panel (*ay* subtype), HBsAg concentrations greater than or equal to 0.03 PEI Units/ml were detected 100% of the time. HBsAg concentrations less than or equal to 0.01 PEI Units/mL were detected 0% of the time. The HBsAg concentration at the cut-off ( $s/c=1.00$ ), estimated from the linear regression, was 0.019 PEI Units/mL (95% CI = 0.008 to 0.029 PEI Units/mL).

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.



The *Vitros* HBsAg Confirmatory Kit was evaluated with the BBI HBsAg Low Titer panel. This panel includes 15 panel members ranging in concentration from 0 - 2.3 ng/mL HBsAg. Two of the panel members (at 0 and 0.7 ng/mL) were observed to be negative for HBsAg (s/c < 1.00) in the *Vitros* HBsAg assay and were not assayed using the *Vitros* HBsAg Confirmatory Kit. All of the panel members (13 of 15) that were observed to be repeatedly reactive in the *Vitros* HBsAg assay were confirmed positive for HBsAg with the *Vitros* HBsAg Confirmatory Kit.

### 9.3. Matrix Effects:

To determine the ability of the *Vitros* HBsAg assay to analyze serum and/or plasma fifty fresh blood samples (25 unspiked and 25 spiked with HBsAg at a target result close to the cut-off), were collected and aliquoted into serum and plasma collection tubes. Sodium heparin, K<sub>2</sub> EDTA, and sodium citrate were evaluated.

For matched HBsAg spiked samples, EDTA compared with serum showed -2.8% mean difference (n=25) in HBsAg s/c ratio. The mean % difference with heparin plasma was observed to be 21.1% lower than serum, and citrate plasma was observed to be 22.2% lower than serum. All of the HBsAg spiked samples tested maintained reactivity in the *Vitros* HBsAg assay regardless of the sample type preparation. All unspiked samples, tested in serum and plasma (EDTA, heparin, and citrate) preparations, were classified correctly as negative.

The 25 samples spiked with HBsAg collected and dispensed into serum and plasma collection tubes (sodium heparin, K<sub>2</sub> EDTA, sodium citrate) were tested in the *Vitros* HBsAg Confirmatory kit. For all samples, the non-neutralized results were all  $\geq 0.80$  s/c (0.85 to 2.21) and the neutralization results ranged from 75.9% to 95.6%. All samples regardless of sample type were classified correctly as positive.

To Compare the stability of serum/plasma samples twenty fresh blood samples (10 unspiked and 10 spiked with HBsAg at a target result close to the cut-off), were collected and aliquoted into serum and plasma collection tubes (K<sub>2</sub>EDTA, sodium citrate, and sodium heparin). Testing with the *Vitros* HBsAg assay was conducted on the same day blood was drawn, and again after 5 and 7 days storage at 2 – 8 °C and after 28 days at -20 °C.

The data showed that storage of serum or plasma (heparin, K<sub>2</sub>EDTA or sodium citrate) samples for up to 5 days at 2 – 8 °C, or 28 days at -20 °C would not have a significant effect on the test results with the *Vitros* HBsAg assay.

The 10 HBsAg spiked samples were tested in serum and plasma, (K<sub>2</sub> EDTA, sodium citrate, heparin). Testing was done with the *Vitros* HBsAg Confirmatory kit on the same day blood was drawn, after 5 and 7 days storage at 2 – 8 °C and after 28 days at -20 °C. For all samples tested, the non-neutralized results were  $\geq 0.80$  and the neutralization results ranged from 79.7% - 100%. All samples regardless of sample type or storage condition were classified correctly as positive.

#### 9.4. Analytical Specificity (Cross-Reacting and Interfering Substances):

To assess the *Vitros* HBsAg assay for potentially cross-reacting biological substances the specificity of the *Vitros* HBsAg assay was evaluated by testing 244 samples from 17 potentially cross-reacting diseases. Patient samples from the following diseases were tested: HAV, HCV, HEV, non-viral liver disease, autoimmune disease (rheumatoid arthritis and systemic lupus erythematosus), CMV, EBV, HSV, parvovirus B19 infection, rubella, syphilis, toxoplasmosis, HIV 1/2 antibody positive, HTLV 1/2 antibody positive, heterophilic antibodies, and recent influenza vaccine recipients. The presence of the analyte of interest was determined by the detection of specific antibody. In addition, samples from 50 blood donors were tested in the study.

Of the 244 samples tested 243 were observed to be negative. One patient sample with HAV infection was found to be false reactive in the *Vitros* HBsAg assay. One of the fifty blood donor samples was observed to be false reactive in the *Vitros* assay.

The following table shows a summary of the results.

Clinical Category	Number Samples Tested	<i>Vitros</i> HBsAg Assay Result < 1.00	<i>Vitros</i> HBsAg Assay Result ≥ 1.00
Hepatitis A Infection (HAV)	10	9	1
Hepatitis C Infection (HCV)	10 <sup>†</sup>	10	0
Hepatitis E Infection (HEV)	10	10	0
Non-viral Liver Disease <sup>‡</sup>	50	50	0
Autoimmune Diseases (Rheumatoid Arthritis / Systemic Lupus Erythematosus)	60	60	0
Cytomegalovirus (CMV)	7	7	0
Epstein-Barr Virus (EBV)	10	10	0
Herpes Simplex Virus (HSV) (HSV1 and HSV2 not distinguished)	10	10	0
Parvovirus B19 Infection	10	10	0
Rubella	11	11	0
Syphilis	16	16	0
Toxoplasmosis	10	10	0
Human Immunodeficiency Virus (HIV 1/2)	10	10	0
Human T-cell Lymphotropic Virus (HTLV 1/2)	10	10	0
Heterophilic Antibodies (Human anti-mouse)	5	5	0
Recent Influenza Vaccine Recipients	10	10	0
Total Samples Tested	249	248	1

\*Classified as falsely reactive. The sample did not demonstrate the ≥ 50% neutralization required to be classified as positive.

<sup>†</sup>Two of these samples were repeatedly Anti-HCV reactive by EIA and strip immunoblot (SIA) positive.

<sup>‡</sup>Samples were obtained from individuals with elevated liver enzymes, alcoholic liver disease, and liver cancer

The specificity of the *Vitros* HBsAg assay was evaluated further by testing samples containing the following potentially cross-reacting material: HCV (bDNA positive), HIV 1 (PCR positive), and HIV 2 (antibody positive). Additionally, testing was performed on serum samples spiked with *Toxoplasma gondii* tachyzoites (whole and sonicated), *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Samples were tested with and without an additional spike of HBsAg. The spiked and unspiked samples were assayed in triplicate. Of the samples tested, none of the unspiked samples were observed to be false reactive and none of the HBsAg spiked samples were observed to be false negative in the *Vitros* HBsAg assay.

The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 10 blood donors as recommended by the National Committee of Clinical Laboratory Standards Protocol EP7-P. The results (mean of test results at each level of interferent) demonstrated 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. HBsAg spiked samples were tested near the *Vitros* HBsAg cut-off, and were observed to remain reactive at all levels tested with each potential interferent. Similarly, no interference was observed in negative samples with no HBsAg added. When testing HBsAg negative

samples for potential interference with triolein, one donor sample was observed to be reactive when ethanol was added to the sample even in the absence of triolein.

The potentially interfering effect of hemoglobin, bilirubin and triolein were evaluated in the *Vitros* HBsAg Confirmatory kit using samples from 10 blood donors spiked with HBsAg at a concentration near the *Vitros* HBsAg cut-off. The individual results and mean results (percent neutralization) 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. For all samples the non-neutralized results ranged from 0.98 to 2.08 and the neutralization results ranged from 66.3 % - 96.4%.

To determine if the assay showed a high dose hook effect, an HBsAg dilution series with concentrations ranging from 0 to 498 µg HBsAg/mL was assayed in the *Vitros* HBsAg assay. Single determinations of each sample were made with each of three Kit Lots. Each additional sample (Dilutions 1-9) in the HBsAg dilution series gave clearly reactive results in the *Vitros* HBsAg assay with each of the Kit Lots tested. This shows that the high dose hook effect does not interfere with the qualitative determination of HBsAg in samples up to 498 µg HBsAg/mL. This was further verified by testing 10 patient samples, which had previously demonstrated, by dilution, a high dose hook effect. All of these samples gave clearly reactive results.

To demonstrate that a high sample dilution is appropriate for specimens with results > 500 s/c, a total of 49 samples with results >500 s/c in the *Vitros* HBsAg assay were diluted 1/151 in Sample Diluent prior to testing with the *Vitros* HBsAg Confirmatory Kit. Of the 49 samples examined in this study, neutralization results ranged from 98.4 to 100%. There were no discrepant samples; all (49/49) were confirmed HBsAg positive with the *Vitros* HBsAg Confirmatory Kit demonstrating satisfactory performance when used with patient samples that require dilution.

#### 9.5. Reagent Stability and Reagent Storage Conditions:

To determine the stability (shelf life) of the *Vitros* HBsAg Reagent Packs, Calibrators and Controls were subjected to a period of simulated transport to mimic effects of shipment. The devices were tested at various time points up to 52 weeks after storage at 2 – 8 °C. In addition, a commercially obtained Hepatitis B seroconversion panel (subtype *ad*) consisting of five panel members were tested using transported, stored materials at week 0, 26, and 52. Trending limits were established to assess deviation of s/c values. Materials stored for 52 weeks yielded results that indicated no change in the classification of the samples (reactive and non-reactive) from the classifications obtained at the initial time point or exceeded the established trending limits.

Stability of the *Vitros* HBsAg Reagent Packs, Calibrators and Controls and Confirmatory Kits were determined by subjecting the devices to a period of simulated transport to mimic effects of shipment. The devices were tested at various time points up to 52 weeks after storage at 2 – 8 °C and the results subjected to established trending limits. This data supports the storage of the *Vitros* HBsAg Confirmatory Kit for 52 weeks at 2 – 8 °C.

Open On-Board Storage for the *Vitros* HBsAg Reagent Pack was determined by subjecting the device to a period of simulated transport to mimic the effects of shipment. *Vitros* HBsAg Reagent Packs were opened and placed in an environmental chamber (4 - 8 °C, ≤ 40% relative humidity) for a period of 8 weeks to simulate storage on-board the *Vitros* Analyzer. These Reagent Packs were tested at various time points within the 8-week period. In addition, an opened Reagent Pack from each kit lot was removed from the chamber and brought to room temperature on 6 different occasions over the 8 week period to simulate typical customer usage. Results of testing were within acceptability limits and overall, no trend was observed between freshly opened Reagent Packs stored at 2 – 8 °C and Reagent Packs stored opened on-board for 8 weeks. This data supports the on-board storage of Reagent Packs for up to 8 weeks.

Open Off-Board Storage for the *Vitros* HBsAg Calibrator was determined by subjecting the device to a period of simulated transport to mimic effects of shipment, *Vitros* HBsAg Calibrators were opened, pooled, sub-aliquoted and stored at 2 – 8 °C and at –20 °C for a period of 13 weeks. Results of testing these Calibrators at various time points up to 13 weeks indicated no observable trends and met all acceptance criteria. This data supports the storage of the Calibrators at 2 – 8 °C and at –20 °C after opening for up to 12 weeks.

Open Off-Board Storage of the Sample Diluent and Confirmatory Antibody in the *Vitros* HBsAg Confirmatory Kit was determined by subjecting the devices to a period of simulated transport to mimic the effects of shipment. The *Vitros* HBsAg Confirmatory Antibody was reconstituted and transferred to sample cups and then stored at 2 – 8 °C for 8 weeks for 12 weeks. The *Vitros* HBsAg Sample Diluent was opened and transferred to sample cups and then stored at 2 – 8 °C for 12 weeks. The stored Confirmatory Antibody and Sample Diluent were compared against freshly reconstituted Confirmatory Antibody and freshly opened Sample Diluent using two Kit lots of *Vitros* HBsAg reagents. The data showed that there were no differences between the fresh and 'open' stored Sample Diluent and fresh and 'open' stored reconstituted Confirmatory Antibody. No trends were evident with the stored Confirmatory Antibody and Sample Diluent. The data supports the storage of the Confirmatory Antibody after reconstitution for up to 8 weeks at 2 - 8 °C. The data also supports the storage of the Sample Diluent at 2 – 8 °C after opening for up to 12 weeks.

*Vitros* HBsAg assay reagents and *Vitros* HBsAg Confirmatory Kit reagents are formulated with anti-microbial agents (Kathon and Bronidox) that provide protection against adventitious contamination by microorganisms. Evaluation of the microbial load of each reagent (Assay Reagent, Conjugate Reagent, Calibrator, Confirmatory Antibody and Sample Diluent) post-dispensing and at expiry (52 weeks) demonstrated that the total aerobic count is generally in the order of ≤ 30 CFU/mL. In addition, the levels of preservative in each reagent were determined over a period of 52 weeks of storage. Results demonstrated that the preservative concentrations in each reagent were above the minimum inhibitory concentration throughout the 52 weeks.

A study conducted according to US Pharmacopoeia (USP) 23/NF 18; general chapter 51 assessed the ability of the reagents to withstand or control microbial contamination. Results indicated that the preservative systems for each reagent met the requirements of USP 23 at 52 weeks based upon the date of manufacture.

#### 9.6. Assay Precision and Reproducibility

Precision was evaluated on different *Vitros* ECI Systems 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.

	Mean <i>Vitros</i> HBsAg S/C (Ratio)	Within Day *		Between Day†		Total ‡		No.	No. Observ. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Site 1	0.11	0.015	14.5	0.023	21.6	0.027	26.0	42	20
	0.75	0.024	3.2	0.035	4.7	0.043	5.7	39	20
	3.05	0.123	4.0	0.239	7.8	0.269	8.8	42	20
Site 2	0.11	0.014	12.4	0.029	25.7	0.032	28.5	40	20
	0.84	0.019	2.3	0.038	4.6	0.043	5.1	40	20
	3.17	0.047	1.5	0.063	2.0	0.079	2.5	40	20
Site 3	0.13	0.028	21.2	0.015	11.3	0.032	24.0	41	20
	0.84	0.022	2.6	0.051	6.0	0.055	6.6	40	20
	3.10	0.048	1.5	0.084	2.7	0.097	3.1	41	20

\* Within Day: Variability of the assay performance from replicate to replicate.

† Between Day: Variability of the assay performance from day to day.

‡ Total: Variability of the assay performance combining the effects of within day and between day.

Precision was further evaluated incorporating between site and between lot variation. The study was performed at three external sites using three different 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.

Mean <i>Vitros</i> HBsAg S/C (Ratio)	Between Site *		Between Lot †		Total ‡		No. Observ.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
0.87	0.058	6.7	0.055	6.4	0.094	10.8	270
0.93**	0.057**	6.2**	0.048**	5.1**	0.090**	9.7**	269**
1.07	0.066	6.2	0.051	4.8	0.106	9.8	270
4.06	0.049	1.2	0.183	4.5	0.249	6.1	270

\* Between site: Variability of the assay performance from site to site.

† Between lot: Variability of the assay performance from lot to lot, calculated using data across all sites.

‡ Total: Variability of the assay incorporating factors of site, lot and day.

\*\* One gross outlier > 21 S. D. from the mean) in 270 observations (0.37%) was excluded from this calculation.

### 9.7. Calibration Interval

The performance of the *Vitros* HBsAg assay within one calibration interval (28 days) was evaluated at three sites by testing a three-member panel with one kit lot. One panel member was close to the *Vitros* HBsAg assay cutoff. Two replicates of each panel member were run per day at each clinical site. Appropriate calibration was performed and verified on day one of the study, and the testing was performed for a total of 20 study days over one calibration interval (28 days).

Least square regression analyses were performed within site and across sites. For analyses within site, the slope was not statistically significant for the panel member closest to the cutoff. For analyses across sites, the slopes were not statistically significant for any of the three panel members, demonstrating adequate performance throughout the entire calibration interval.

### 9.8. Seroconversion Panels

Seventeen HBV seroconversion panels were obtained from two commercial vendors. 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 the 17 panels with the *Vitros* and reference HBsAg assays.

Days to HBsAg Reactive Result from Initial Draw Date			
Panel	Reference HBsAg Assay	Vitros HBsAg Assay	Difference in Days to HBsAg Reactive Result (Reference Vitros)
1	13	13	0
2	26	19	7
3	37	29	8
4	50	50	0
5	45	45	0
6	40	36	4
7	68	68	0
8	21	14	7
9	36	36	0
10	21	21	0
11	34	27	7
12	35	35	0
13	15	23	-8
14	26	26	0
15	0	0	0
16	16	16	0
17	9	7	2

## 10. Summary of Clinical Studies

### 10.1. *Vitros* HBsAg 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.

Age Range	Gender	Vitros HBsAg Result				Total
		+		-		
		n	Percent	n	Percent	
0-9	F	0	NA	0	NA	0
	M	0	NA	1	100	1
10-19	F	2	11	16	89	18
	M	0	NA	11	100	11
20-29	F	1	1	122	99	123
	M	2	2	110	98	112
30-39	F	1	1	149	99	150
	M	18	8	214	92	232
40-49	F	3	2	154	98	157
	M	10	4	235	96	245
50-59	F	3	3	106	97	109
	M	4	4	101	96	105
60-69	F	0	NA	67	100	67
	M	3	7	42	93	45
70-79	F	0	NA	27	100	27
	M	0	NA	26	100	26
80-89	F	0	NA	5	100	5
	M	0	NA	2	100	2
90-100	F	0	NA	0	100	0
	M	0	NA	1	100	1
<b>Total</b>		47		1389		1436*

\*Age was not reported for three subjects.

#### 10.2. Vitros HBsAg 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 the 2156 of 2177 subjects enrolled. 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 markers). These 21 subjects were excluded from the analysis.

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.

HBsAg <sup>***</sup>	HBeAg	HBV Reference Markers				HBV Classification
		IgM Anti-HBc	Total Anti-HBc	Anti-HBe	Anti-HBs (≥10 mIU/mL)	
+	+	+	+	+	-	Acute
+	+	+	+	-	-	Acute
+	-	+	+	+	-	Acute
+	-	-	-	-	-	Acute
+	+	-	+	+	-	Chronic
+	+	-	+	-	-	Chronic
+	-	-	+	+	+	Chronic
+	-	-	+	+	-	Chronic
+	-	-	+	-	-	Chronic
-	-	+	+	+	+	Early Recovery
-	-	+	+	+	-	Early Recovery
-	-	+	+	-	+	Early Recovery
-	-	+	+	-	-	Early Recovery
-	-	-	+	+	-	Early Recovery
-	-	-	+	+	+	Recovery
-	-	-	+	-	+	Recovered
-	-	-	+	-	-	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected
+	-	-	-	-	+	Uninterpretable
-	+	-	+	-	-	Uninterpretable
-	+	-	-	-	+	Uninterpretable
-	+	-	-	-	-	Uninterpretable
-	-	+	-	-	-	Uninterpretable

\*\*\*Positive (+) = Reference HBsAg assay reactive and confirmed by neutralization. Negative (-) = Reference HBsAg assay negative or not confirmed by neutralization.

The table below compares the *Vitros* HBsAg results with the reference HBsAg results by specimen classification for the prospective sample population. The data in the table are representative of the number of specimens in each result category.

Disease Classification	Final Reference HBsAg Assay Result <sup>1</sup>				Total
	- <sup>2</sup>		+		
	Final <i>Vitros</i> HBsAg Result				
	-	+	-	+	
Acute infection	0	0	5 <sup>3</sup>	20	25
Chronic infection	0	0	2	51	53
Early recovery	64	1	0	0	65
Recovery	184	0	0	0	184
Recovered	266	2	0	0	268
Uninterpretable	11	0	1	1	13
HBV vaccine response	236	0	0	0	236
Not previously infected with HBV	1304	8	0	0	1312
<b>Grand Total</b>	<b>2065</b>	<b>11</b>	<b>8</b>	<b>72</b>	<b>2156</b>

- <sup>1</sup> Final reference HBsAg assay result is based on the initial test result and confirmatory testing of repeatedly reactive samples.
- <sup>2</sup> 14 specimens were reactive with the initial reference HBsAg assay, but did not confirm (3 - Recovery, 2 - HBV vaccine response, 9 - Not previously infected with HBV). All other specimens were nonreactive with the initial reference HBsAg assay.
- <sup>3</sup> These five samples were positive only for the reference HBsAg assay. Four of these 5 patients had normal ALT levels and showed no clinical signs or symptoms of HBV infection. Their clinical presentation was not consistent with the results of the reference HBsAg assay. Taking the clinical symptoms and normal ALT levels into consideration the negative *Vitros* HBsAg result appears to be more consistent with their clinical presentation. The remaining sample where HBsAg was the only marker detectable also showed elevated ALT levels and was reference assay anti-HCV positive.

The following table shows the results for the *Vitros* HBsAg cutoff between  $\geq 1.00$  and  $\leq 5.00$  that requires repeat testing, and if repeatedly reactive, confirmation testing by the *Vitros* HBsAg Confirmatory Assay.

**Samples with Two of Three *Vitros* HBsAg Initial and Repeat Test Results  $\geq 1.00$  and  $\leq 5.00$**

Disease Classification	Final Reference HBsAg Assay Result <sup>1</sup>						Total
	+			-			
	Final <i>Vitros</i> HBsAg Result						
	-	+	NT <sup>2</sup>	-	+	NT <sup>2</sup>	
Acute infection	0	0	1	0	0	0	1
Chronic infection	0	1	0	0	0	0	1
Early recovery	0	0	0	1	0	0	1
Not previously infected with HBV	0	0	0	1	2	0	3
<b>Grand Total</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>6</b>

- <sup>1</sup> Final reference HBsAg assay result is based on the initial test result, repeat test results and confirmatory testing of repeatedly reactive samples.
- <sup>2</sup> *Vitros* HBsAg Confirmatory Testing was not performed.

The following table shows the results for the *Vitros* HBsAg cutoff of  $> 5.00$  where confirmatory testing with the *Vitros* HBsAg Confirmatory assay is not required.

**Samples with Initial or 2 of 3 *Vitros* HBsAg S/C Results  $> 5.00$  where *Vitros* HBsAg Confirmatory Testing was not Required**

Disease Classification	Final Reference HBsAg Assay Result <sup>1</sup>		Total
	+	- <sup>2</sup>	
	Final <i>Vitros</i> HBsAg Result		
	+	+	
Acute infection	19	0	19
Chronic infection	50	0	50
Recovered	0	2	2
Uninterpretable	1	0	1
Not previously infected with HBV	0	5	5
<b>Grand Total</b>	<b>70</b>	<b>7</b>	<b>77</b>

- <sup>1</sup> Final reference HBsAg assay result is based on the initial test result, repeat test results and confirmatory testing of repeatedly reactive samples.
- <sup>2</sup> Initial reference HBsAg assay result was negative.

The table below summarizes the percent agreement between the *Vitros* HBsAg assay and the HBsAg reference assay for each specimen classification for the prospective sample population supplemented with archived reference HBsAg positive samples. The table provides the upper and lower 95% exact confidence bounds.

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Overall	90.0 (72/80)	81.2 to 95.6	99.5 (2065/2076)	99.1-99.7
Acute	80.0 (20/25)	59.3 to 93.2	NA	NA
Chronic	96.2 (51/53)	87.0 to 99.5	NA	NA
Early Recovery	NA	NA	98.5 (64/65)	91.7 to 100
Recovery	NA	NA	100 (184/184)	98.0 to 100
Recovered	NA	NA	99.3 (266/268)	97.3 to 99.9
Uninterpretable	50.0 (1/2)	1.3 to 98.7	100 (11/11)	71.5 to 100
HBV Vaccine Response	NA	NA	100 (236/236)	98.5 to 100
Not Previously Infected	NA	NA	99.4 (1304/1312)	98.8 to 99.7

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 HBV infection, (e.g., HBsAg present  $\geq$  6 months). Samples were obtained from commercial and site archives. Archived samples from dialysis patients demonstrating seroconversion to HBsAg positive status were included.

The table below summarizes the overall clinical performance of the *Vitros* HBsAg assay in samples from subjects with documented acute or chronic HBV infection.

HBV Infection	Number of Samples	Number (%) of <i>Vitros</i> HBsAg Positive Samples	95% Exact Confidence Interval
Acute	38	36 (94.7)	82.3 to 99.4
Chronic	32	32 (100.0)	88.4 to 100
Total	68	66 (97.1)	89.8 to 99.6

\*The two acute samples negative by the *Vitros* HBsAg assay were obtained from patients undergoing seroconversion to HBsAg positive status. Occasional discrepancies may occur between different manufacturer's HBsAg assays when testing samples during early seroconversion.

### 10.3. *Vitros* HBsAg 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 a 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 shows the demographic profile of the pregnant women studied:

Demographic Profiles of Pregnant Women at Low or High Risk for Viral Hepatitis			
RISK	LOW N (%)	HIGH N (%)	TOTAL N (%) <sup>1</sup>
<b>TOTAL<sup>2</sup></b>	463 (62.2)	281 (37.8)	744 (100)
<b>TRIMESTER</b>			
First	200 (43.2)	67 (23.8)	267 (35.9)
Second	152 (32.8)	102 (36.3)	254 (34.1)
Third	111 (24.0)	112 (39.9)	223 (30.0)
<b>ETHNICITY<sup>3</sup></b>			
Caucasian	269 (58.1)	17 (6.0)	286 (38.4)
African-American	53 (11.4)	75 (26.7)	128 (17.2)
Hispanic	125 (27.0)	158 (56.2)	283 (38.0)
Asian	7 (1.5)	1 (0.4)	8 (1.1)
Indian	1 (0.2)	1 (1.4)	5 (0.7)
Haitian	1 (0.2)	6 (5.7)	17 (2.3)
Other	7 (1.5)	6 (2.1)	13 (1.7)
Unknown	0(0)	4 (1.4)	4 (0.5)
<b>AGE (Years)</b>			
11-30	268 (57.9)	182 (64.8)	450 (60.5)
31-50	194 (41.9)	99 (35.2)	293 (39.4)
Unknown	1 (0.2)	0(0)	1 (0.1)

<sup>1</sup>The total number (N) of subjects at both low and high risk belonging to the variable category in the left hand column; expressed as a percentage (%) of all analyzed subjects (N=744).

<sup>2</sup>The total number (N) of subjects at high risk; expressed as a percentage (%) of all analyzed subjects (N=744).

<sup>3</sup>The number (N) of subjects at low or high risk belonging to the variable category in the left hand column; expressed as a percentage (%) of all subjects at low or high risk.

The tables below compare the *Vitros* and reference HBsAg assays among the overall population of pregnant women by risk and trimester.

***Vitros* and Reference HBsAg Results Among Low Risk Pregnant Women**

<i>Vitros</i> HBsAg Result	First Trimester			Second Trimester			Third Trimester		
	Reference HBsAg Result		Total	Reference HBsAg Result		Total	Reference HBsAg Result		Total
	+	-		+	-		+	-	
+	2	0	2	0	0	0	0	0	
-	0	198	198	0	152	152	0	111	
Total	2	198	200	0	152	152	0	111	

***Vitros* and Reference HBsAg Results Among High Risk Pregnant Women**

<i>Vitros</i> HBsAg Result	First Trimester			Second Trimester			Third Trimester		
	Reference HBsAg Result		Total	Reference HBsAg Result		Total	Reference HBsAg Result		Total
	+	-		+	-		+	-	
+	1	0	1	1	0	1	1	0	
-	0	66	66	0	101	101	0	111	
Total	1	66	67	1	101	102	1	111	

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.

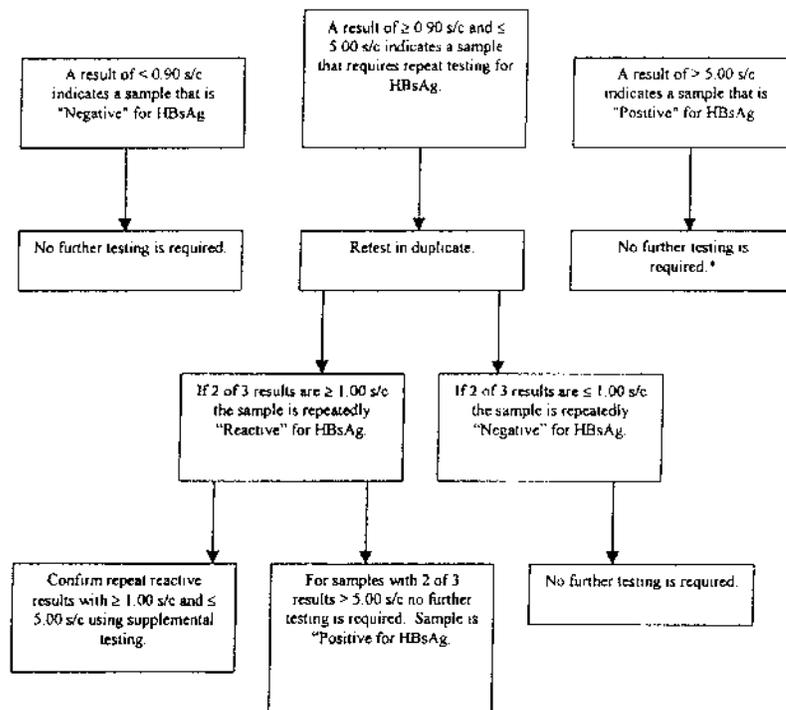
<i>Vitros</i> HBsAg Result	Reference HBsAg Result		Total N (%)
	+ N (%)	- N (%)	
+	5 (100)	0 (0.0)	5 (0.7)
-	0 (0.0)	739 (100)	739 (99.3)
Total	5 (0.7)	739 (99.3)	744 (100)

The table below summarizes the percent agreement between the *Vitros* HBsAg assay and the HBsAg reference assay for this population. The table provides the upper and lower 95% confidence exact confidence bounds.

Subjects	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Pregnant Women	100 (5/5)	47.8 to 100	100 (739/739)	99.5 to 100

#### 10.4. Interpretation of *Vitros* HBsAg Assay Results:

Based on the preclinical and clinical laboratory study information presented, the following *Vitros* HBsAg assay result interpretation was established.



**Interpretation of Results**

Vitros HBsAg Assay Result (s/c)	Conclusion from Testing Algorithm	Interpretation
< 1.00	Negative	Specimen is presumed to be negative for HBsAg.
≥ 1.00 and ≤ 5.00	Reactive	Patient is reactive for HBsAg. If a repeat reactive result is confirmed by supplemental tests, such as the <i>Vitros</i> Immunodiagnostic Products HBsAg Confirmatory Kit, specimen is positive for HBsAg.
> 5.00	Positive	Specimen is positive for HBsAg.*

\* In instances where HBsAg is used as a stand alone assay (for example in pregnant women being screened to identify neonates who are at risk for acquiring HBV during the perinatal period), it is recommended that supplemental testing such as the *Vitros* HBsAg Confirmatory Kit be used to confirm the result.

10.5. Assay Performance Characteristics for The *Vitros* HBsAg Confirmatory Kit:

*Vitros* Confirmatory assay performance characteristics were established using reference HBsAg positive samples from various sources. All samples were reactive by the reference HBsAg assay and confirmed positive by neutralization using the reference HBsAg confirmatory kit.

The performance of the *Vitros* HBsAg Confirmatory Kit was evaluated among three groups of samples:

- A prospective sample population.
- Archived commercial samples historically HBsAg reactive by the reference HBsAg assay and confirmed by neutralization using the reference confirmatory kit.
- HBsAg positive samples from pregnant women, archived samples from dialysis patients, and archived samples from acute or chronic HBV infections.

The following two tables summarized the performance of the *Vitros* HBsAg Confirmatory Kit on samples from the prospective population where *Vitros* HBsAg results were repeatedly reactive (s/c ≥ 1.00 and ≤ 5.00) or HBsAg positive (≥ 5.00).

***Vitros* HBsAg Confirmatory Testing in Repeatedly Reactive Samples with Two of Three Initial and Repeat Test Results  $\geq 1.00$  and  $\leq 5.00$**

Disease Classification	Final Reference HBsAg Assay Result				Total
	+ <sup>1</sup>		- <sup>2</sup>		
	<i>Vitros</i> HBsAg Confirmatory Testing				
	+	NT <sup>3</sup>	+	-	
Acute infection	0	1	0	0	1
Chronic infection	1	0	0	0	1
Early recovery	0	0	0	1	1
Not previously infected with HBV	0	0	2	1	3
<b>Grand Total</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>6</b>

- <sup>1</sup> Final reference HBsAg assay result is based on the initial test result, repeat test results and confirmatory testing of repeatedly reactive samples.
- <sup>2</sup> Reference HBsAg initial screening was negative; confirmatory testing was not performed.
- <sup>3</sup> Specimen was repeatedly reactive with the *Vitros* HBsAg assay; *Vitros* HBsAg Confirmatory Testing was not performed.

**Samples with Initial or 2 of 3 Repeat *Vitros* HBsAg S/C Results  $> 5.00$**

Disease Classification	Final Reference HBsAg Assay Result			Total
	+ <sup>1</sup>		- <sup>2</sup>	
	Final <i>Vitros</i> HBsAg Result			
	+	+	-	
Acute infection	3	0	0	3
Chronic infection	2	0	0	2
Uninterpretable	1	0	0	1
Not previously infected with HBV	0	1	1	2
<b>Grand Total</b>	<b>6</b>	<b>1</b>	<b>1</b>	<b>8</b>

- <sup>1</sup> Final reference HBsAg assay result is based on the initial test result and confirmatory testing of repeatedly reactive samples.
- <sup>2</sup> Reference HBsAg initial screening was negative; confirmatory testing was not performed.

The table below summarizes the various sources and numbers of samples evaluated using the *Vitros* HBsAg Confirmatory Kit. An HBsAg positive result by neutralization was obtained in 82 of 82 (100%) samples using the *Vitros* HBsAg Confirmatory Kit on HBsAg reference assay reactive and confirmed positive samples.

Sample Sources	N	Reference HBsAg Reactive and Confirmed Positive by Neutralization	<i>Vitros</i> HBsAg Confirmatory Kit Confirmed Positive by Neutralization
Individuals with signs or symptoms of hepatitis or at high risk for HBV infection	7	7	7
Known HBsAg positive archived samples	48	48	48
Individuals with early acute infection	21	21	21
Individuals with clinically diagnosed chronic infection	1	1	1
Pregnant females	5	5	5
<b>Total</b>	<b>82</b>	<b>82</b>	<b>82 (100%)</b>

The table below summarizes the distribution of *Vitros* HBsAg results and corresponding *Vitros* HBsAg Confirmatory Kit results among the combined 82 samples obtained from the various sources listed above.

<i>Vitros</i> HBsAg Assay Result (s/c)	N	Number (%) Confirmed in the <i>Vitros</i> HBsAg Confirmatory Kit
≥ 0.90 and < 5.00	10	10 (100)
≥ 5.00 and < 10.0	7	7 (100)
≥ 10.0 and < 20.0	11	11 (100)
≥ 20.0 and < 100	13	13 (100)
≥ 100 and < 500	9	9 (100)
≥ 500	32	32 (100)
<b>Total</b>	<b>82</b>	<b>82 (100)</b>

The following tables show a summary of the interpretation of results for the *Vitros* HBsAg Confirmatory Kit.

All samples		
Non-neutralized result (s/c)	% Neutralization	Confirmatory Test Result Classification/Action to be taken
≥ 0.80	≥ 50%	Confirmed (HBsAg positive)
≥ 0.80	< 50%	Dilute further and retest
< 0.80	Any value	Retest

Samples after dilution and retest		
Non-neutralized result (s/c)	% Neutralization	Confirmatory Test Result Classification/Action to be taken
≥ 0.80	≥ 50%	Confirmed (HBsAg positive)
> 100	< 50%	Dilute further and retest
100	< 50%	Not confirmed (HBsAg negative)

## 11. Conclusions Drawn from Studies

The preclinical and clinical study data demonstrates that acceptable performance is obtained with the *Vitros* HBsAg assay and *Vitros* HBsAg Confirmatory Kit when testing specimens collected in serum or heparin, K<sub>2</sub>EDTA, and sodium citrate anticoagulated plasma.

The *Vitros* HBsAg Reagent Pack, Calibrator, and Confirmatory Kit can be stored up to 52 weeks at 2 – 8 °C, while the Reagent Pack can be stored on-board the *Vitros* Analyzer (4 - 8 °C, ≤40% relative humidity) for up to 8 weeks, and the Calibrator stored for up to 13 weeks at 2 – 8 °C after opening.

The *Vitros* HBsAg Confirmatory Antibody may be stored after reconstitution for up to 8 weeks at 2 – 8 °C and 12 weeks at –20 °C. The data also supports the storage of the Sample Diluent at 2 – 8 °C and –20 °C after opening for up to 12 weeks.

The preservative systems used in the *Vitros* HBsAg assay and Confirmatory reagents are formulated with antimicrobial agents that have been shown to meet USP 23 requirements at

52 weeks for the Assay Reagent, Conjugate Reagent, Calibrator, Confirmatory Antibody, and Sample Diluent.

The *Vitros* HBsAg assay demonstrated acceptable precision estimates for within day and between day for each site as well as across all sites, and between replicate, between day, between site, and between lot when these variables were introduced.

The *Vitros* HBsAg assay has been shown to perform adequately over a 28-day calibration interval.

The *Vitros* HBsAg Confirmatory Kit demonstrated the ability to confirm the presence of HBsAg in positive samples from a variety of sources across a broad range of s/c results.

### **Safety**

As a diagnostic test, the HBsAg assay involves removal of blood from an individual for testing purposes. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood is removed.

### **Benefit/Risk**

The submitted studies have shown that the *Vitros* Immunodiagnostic Products HBsAg Reagent Pack and Confirmatory Kit, when compared to reference laboratory procedures, has a similar ability to detect the presence of HBsAg in specimens from individuals acutely and chronically infected with HBV. The rate of false positivity and false negativity are within acceptable limits compared to the reference assay. It has been shown that the device has no demonstrable cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, the devices should benefit the physician in the diagnosis of HBV associated and nonassociated hepatitis. The devices will also aid in the identification of pregnant women infected with HBV.

Based on the results of the preclinical and clinical laboratory studies the *Vitros* Immunodiagnostic Products' HBsAg assays, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and effective and pose minimal risk to the patient due to false test results.

## **12. CDRH Decision**

FDA issued an approval order on \_\_\_\_\_.

The applicant's manufacturing facility was inspected on February 15, 2000 and was found to be in compliance with the device Good Manufacturing Practice regulation.

## **13. Approval Specifications**

Directions for use: See Labeling

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the approval order. **APR 27 2001**

Postapproval Requirements and Restrictions: See approval order.