SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:
Antibody to Hepatitis B e Antigen Assay (Anti-HBe)
Antibody to Hepatitis B e Antigen Controls

Device Trade Name:
VITROS Immunodiagnostic Products Anti-HBe Test (VITROS Anti-HBe)
VITROS Immunodiagnostic Products Anti-HBe Reagent Pack
VITROS Immunodiagnostic Products Anti-HBe Calibrator
VITROS Immunodiagnostic Products Anti-HBe Controls

Applicant's Name and Address:
Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, New York 14626-5101

Date of Panel Recommendation:
None

Premarket Approval Application (PMA) Number:
P100001

Date of FDA Notice of Approval:
July 20, 2011

Expedited:
Not applicable.

II. INDICATIONS FOR USE

VITROS Immunodiagnostic Products Anti-HBe Reagent Pack

For the *in vitro* qualitative detection of antibodies to hepatitis B e antigen (anti-HBe) in human adult and pediatric (2 to 21 years old) serum from individuals who have symptoms of chronic hepatitis and those who have recovered from HBV infection, using the VITROS ECi/ECiQ Immunodiagnostic Systems. Further assessment of HBV infection (biochemical, serological and/or nucleic acid testing) is required to define the specific disease state. VITROS Anti-HBe test performance has not been established for the monitoring of HBV disease or therapy.

VITROS Immunodiagnostic Products Anti-HBe Calibrator

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems when used with the VITROS Anti-HBe test for the *in vitro* qualitative detection of antibodies to hepatitis B e antigen (anti-HBe).

VITROS Immunodiagnostic Products Anti-HBe Controls

For use in monitoring the performance of the VITROS Anti-HBe test when used on the VITROS ECi/ECiQ Immunodiagnostic Systems.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VITROS Immunodiagnostic Products Anti-HBe Reagent Pack, Calibrator and Controls labeling.

V. DEVICE DESCRIPTION

A. Assay Principle

The VITROS Anti-HBe assay is performed using the VITROS Anti-HBe Reagent Pack and the VITROS Anti-HBe Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System. The VITROS ECi/ECiQ Immunodiagnostic System allows for the determination of analytes in human samples utilizing an enhanced chemiluminescence detection reaction.

A competitive assay technique is used which involves pre-incubation of anti-HBe in the sample with a fixed weight of HBeAg in the Assay Reagent, followed by incubation with a Conjugate Reagent that contains biotinylated mouse monoclonal anti-HBe antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBe antibody. The formed immune complex is captured by streptavidin on the reaction wells; unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the VITROS ECi/ECiQ Immunodiagnostic System. The amount of

HRP conjugate bound is indirectly proportional to the concentration of anti-HBe present in the sample.

B. Kit Configuration and Components

a) The VITROS Immunodiagnostic Products Anti-HBe test is comprised of the following:

- VITROS ECi/ECiQ Immunodiagnostic System – dedicated instrumentation, cleared by the FDA as an Immunodiagnostic System (K962919/S1), which provides automated analysis of the VITROS Immunodiagnostic System assays.
- VITROS Immunodiagnostic Products Anti-HBe Reagent Pack (VITROS Anti-HBe Reagent Pack) and VITROS Immunodiagnostic Products Anti-HBe Calibrator (VITROS Anti-HBe Calibrator) together comprise the VITROS Anti-HBe assay.
- VITROS Immunodiagnostic Products Anti-HBe Controls are controls used for monitoring the VITROS Anti-HBe assay.
- VITROS Immunodiagnostic Products Signal Reagent and VITROS Immunodiagnostic Products Universal Wash Reagent are universal reagents used in all VITROS Immunodiagnostic System assays.

b) The VITROS Immunodiagnostic Products Anti-HBe Reagent Pack is composed of three reagents:

- 100 coated wells (streptavidin source, bacterial; binds ≥3ng biotin/well)
- 8.4 mL assay reagent containing treated rHBeAg (4.4 Units* /mL) in buffer with mouse serum and antimicrobial agent
- 6.6 mL conjugate reagent (source, HRP-mouse monoclonal anti-HBe 0.3 μg/mL and biotin-mouse monoclonal anti-HBe 5.0 μg/mL) in buffer with sheep and mouse serum and antimicrobial agent

*Paul-Ehrlich-Institute Reference Serum

c) The VITROS Immunodiagnostic Products Anti-HBe Calibrator contains:

- 3 vials of VITROS Anti-HBe Calibrator (freeze-dried, anti-HBe positive human plasma in HBeAg and anti-HBe negative defibrinated and delipidized human plasma with antimicrobial agent); reconstitution volume 1.0 mL

d) The VITROS Immunodiagnostic Products Anti-HBe Controls contains:

- 3 sets of Controls 1 and 2 (freeze-dried defibrinated and delipidized human plasma with antimicrobial agent), reconstitution volume 1.0 mL.

C. Calibration

The VITROS Anti-HBe test is calibrated using a negative Reference Calibrator which is used to establish a cutoff that has optimum clinical sensitivity and specificity for the assay. The cutoff signal level is assigned a result of 1.00. The assay cutoff is maintained relative to the Reference Calibrator signal and is used to establish a valid calibration specific to each lot of reagents.

Patient sample results are calculated as a normalized signal, relative to the cutoff signal (C/S):

Result = Cutoff Signal / Sample Signal

Assay results ≥1.00 C/S indicate a reactive sample, positive for anti-HBe. Assay results <1.00 C/S indicate a non-reactive sample, negative for anti-HBe. A retest zone between ≥0.80 and <1.20 requires that samples with an initial C/S value within that range to be re-tested in duplicate.

D. Interpretation of results

Results are automatically calculated by the VITROS ECi/ECiQ Immunodiagnostic Systems and patient sample results are displayed with a "Negative", "Retest?" or "Reactive" label. Below is the final result interpretation algorithm:

Initial Test Results (C/S)	Retest	Final Interpretation
<0.80	Not Required	Negative (-)
≥0.80 and <1.20	Retest in duplicate	Sample is non-reactive and presumed negative for anti- HBe if 2 of 3 results are <1.00. Sample is reactive and presumed positive for anti- HBe if 2 of 3 results are ≥ 1.00.
≥1.20	Not Required	Reactive (+)

E. Quality Control

The performance of the VITROS Anti-HBe test is monitored using the VITROS Anti-HBe Controls. The performance of the VITROS Anti-HBe Controls has not been established with any other anti-HBe assay.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other alternatives for the determination of HBV infection and its disease stage. Detection of anti-HBe in patients who may be infected with the hepatitis B virus may also be accomplished by using other commercially available FDA approved serological tests. This assay is one of several hepatitis marker assays that are often used together and in conjunction with clinical assessment and other laboratory test results in the diagnosis of the HBV infection.

VII. MARKETING HISTORY

The VITROS Immunodiagnostic Products Anti-HBe Reagent Pack, Calibrator and Controls are currently marketed in Europe, Asia, North and South America, and Oceania. The device has not been withdrawn to date from the market in any country for reasons relating to the safety and effectiveness of the device. The following table provides the list of countries where the product is distributed currently:

Argentina	Kuwait
Armenia	Kyrgystan
Australia	Latvia
Austria	Lebanon
Azerbaijan	Liberia
Bangladesh	Libya
Belarus	Lichtenstein
Belguim	Lithuania
Bolivia	Luxembourg
Brazil	Malaysia
Brunei	Maldives
Bulgaria	Malta
Burma	Martinique
Canada	Mexico
Chile	Moldova
China	Nepal
Colombia	Netherlands
Costa Rica	New Zealand
Croatia	Nicaragua
Cyprus	Nigeria
Czech Republic	Norway
Denmark	Oman
Dominican Republic	Panama
Ecuador	Paraguay
Egypt	Peru
El Salvador	Philippines
Estonia	Poland
Finland	Portugal
France	Reunion
French Antilles	Romania
French Guayana	Russia
Georgia	Saudia Arabia
Germany	Singapore

Greece	Slovak Republic
Guadeloupe	Slovenia
Guatemala	South Africa
Haiti	Spain
Honduras	Sri Lanka
Hong Kong	Sweden
Hungary	Switerland
Iceland	Taiwan
India	Tajikistan
Indonesia	Thailand
Iran	Trinidad and Tobago
Iraq	Turkey
Ireland	Turkmenistan
Israel	United Arab Emirates
Italy	United Kingdom
Jamaica	Uruguay
Japan	Venezuela
Korea	Vietnam

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects associated with the use of the device.

Since the VITROS Immunodiagnostic Products Anti-HBe assay is for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient. However, failure of the product to perform as indicated, or human error in use of the product may lead to a false result. Anti-HBe antibody is an intermediate or long-term risk analyte; repeatedly erroneous false positive or false negative anti-HBe results could lead to inappropriate initiation or cessation of antiviral therapy.

The risk of incorrect test results is inherent with all *in vitro* diagnostic products. Therefore, the above potential risks are not unusual in the laboratory setting and should be evaluated in conjunction with other clinical indicators.

When used according to the instructions in the package insert, there are no known direct adverse effects of this device on the health of the user. Standard good laboratory practices are considered sufficient to minimize risks to the end user.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Analytical Sensitivity

The sensitivity of the VITROS Anti-HBe assay was assessed by testing a series of dilutions of a Paul-Ehrlich Institute (PEI) Anti-HBe reference serum having a known concentration. A stock solution with an anti-HBe level of 100 PEI U/mL was used to prepare an 11 member dilution series at concentrations ranging from 1.00 to 0.00 PEI U/mL. Each dilution was analyzed at n=3 using two Master Lots of reagents on two VITROS ECi/ECiQ Systems. A linear regression of the mean VITROS Anti-HBe assay

result versus the calculated concentration of each dilution was used to determine the concentration of the cut-off.

The concentration at the cut-off (C/S = 1.00) of the VITROS Anti-HBe assay, as determined from the Linear Regression, was 0.20 PEI Units/mL.

B. Cut-off Determination

The position of the assay cutoff was initially based on experimental data generated on clinical samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System. The samples came from known anti-HBe reactive, seroconversion panels, blood donor samples determined to be negative for anti-HBe using other commercially available methods and clinical samples from routine laboratory testing of patients with diseases clinically related to HBV infection but expected to be negative for anti-HBe. The cut-off signal was then established as that light signal which gave the best discrimination between anti-HBe reactive and anti-HBe negative sample populations, to provide optimum specificity and sensitivity for the assay. The cut-off signal level was assigned a result of 1.00. An assay result $\geq$ 1.00 C/S indicates a reactive sample, positive for anti-HBe. An assay result < 1.00 C/S indicates a non- reactive sample, negative for anti-HBe.

C. Antibody Characterization

The physio-chemical properties of the purified mouse monoclonal anti-HBe antibodies utilized in the VITROS Anti-HBe assay were characterized by isotype determination, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), and isoelectric focusing (IEF) polyacrylamide gel electrophoresis. The investigation of the three lots of mouse monoclonal anti-human IgG antibodies ZAG127 (conjugated to horseradish peroxidase) and of ZAS129 (conjugated to biotin) determined that:

- The isotype of the three lots of ZAG127 and of ZAS129 was IgG2a, kappa.
- The SDS PAGE of the three lots of ZAG127 and of ZAS129 showed similar banding patterns with molecular weights typical of immunoglobulin heavy and light chains.
- The IEF gel electrophoresis of the three lots of ZAG127 and of ZAS129 showed similar banding patterns within part number, with pI values typical of monoclonal antibodies.

D. Potentially Cross Reacting Subgroups

The specificity of the VITROS Anti-HBe assay was evaluated by testing a total of 209 patient samples representing various potentially cross-reacting sub-groups: Hepatitis C Virus (HCV), Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Herpes Simplex Virus (HSV), Rubella, Syphilis, Toxoplasmosis, elevated liver enzymes (ALT), Rheumatoid Factor (RF), anti-nuclear antibodies (ANA), heterophilic anti-mouse antibodies (HAMA), past Hepatitis A infection/immunized (HAV Total), non-viral liver

disease, Autoimmune Disease (RA) Rheumatoid Arthritis, Autoimmune Disease (SLE), Parvovirus B19 infection, HIV 1/2, HTLV 1/2, recent Influenza vaccine recipients, cord blood, and *E.Coli*. The majority of the samples tested in this analytical specificity study were characterized based on the relevant antibody markers; documented clinical diagnosis was used to characterize the samples from the non-viral liver disease, SLE and Autoimmune Disease (RA) Rheumatoid Arthritis sub-groups. The samples were also tested in the FDA approved comparator anti-HBe assay.

Of the 209 samples, 17 were found to be repeatedly reactive in the VITROS Anti-HBe assay. All 17 repeat reactive samples were also positive in the FDA approved comparator anti-HBe assay.

Sample Category	No. of Samples Tested	No. Negative	No. Initial Reactive	No. Repeat Reactive
Hepatitis C Virus Infection (HCV)	10	8	2	2*
Cytomegalovirus (CMV)	10	10	0	0
Epstein Barr Virus (EBV)	10	10	0	0
Herpes Simplex Virus (HSV)	10	10	0	0
Rubella	10	10	0	0
Syphilis	10	8	2	2*
Toxoplasmosis	10	9	1	1*
Elevated Liver Enzymes (ALT)	10	10	0	0
Rheumatoid Factor (RF)	10	9	1	1*
Anti-Nuclear Antibodies(ANA)	10	10	0	0
Heterophilic anti-mouse antibodies (HAMA)	9	8	1	1*
Past Hepatitis A Infection/Immunized (HAV Total)	10	8	2	2*
Non-viral Liver Disease	10	9	1	1*
AutoImmune Disease Rheumatoid Arthritis (RA)	10	10	0	0
AutoImmune Disease (SLE)	10	9	1	1*
Parvovirus B19 Infection	10	6	4	4*
HIV 1/2	10	8	2	2*
HTLV 1/2	10	10	0	0
Recent Influenza Vaccine Recipients	10	10	0	0
Cord Blood	10	10	0	0
Escherichia coli	10	10	0	0

* Also positive in FDA approved comparator assay

E. Interfering Substances

The VITROS Anti-HBe assay was evaluated for interference from common endogenous substances by testing bilirubin, triolein, biotin, dipyrone, and hemoglobin, as recommended by the Clinical and Laboratory Standards Institute document EP7-A2. All interfering substance evaluations were performed using patient samples. Performance was assessed in both negative and positive samples for anti-HBe. Samples were tested in triplicate using two master lots of reagents on two VITROS ECi/ECiQ Immunodiagnostic Systems. Bilirubin, triolein, biotin and dipyrone were found not to interfere with the assay up to the concentrations indicated below:

Compound	Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Biotin	40.8 nmol/L	1 µg/dL
Triolein	33.9 mmol/L	3000 mg/dL
Dipyrone	3.0 mmol/L	100 mg/dL

Hemoglobin, when tested, was found to interfere with the VITROS Anti-HBe results. At concentrations > 125 mg/dL the observed bias in the results was -26.9%.

Interferent	Interferent Concentration		Units = C/S	
			Mean Result*	Bias**
Hemoglobin	0.076 mmol/L	125 mg/dL	1.39***	-26.9%

*Mean result of replicate determinations using 2 different lots of reagent
**Estimate of the average difference observed
***Reactive sample showed negative bias

The effect of elevated serum protein levels on the VITROS Anti-HBe test was not evaluated. Each clinical laboratory should verify the performance of this test with samples with high protein content in accordance with CLSI document EP7-A2.

F. Serum Sample Stability

The effect of temperature on the integrity of the anti-HBe antibody in serum samples was evaluated at 2-8 °C, at -20 °C and at room temperature (21 °C and 30 °C). Ten (10) blood samples from 10 anti-HBe negative patients were spiked with anti-HBe positive plasma to a level close to the cut-off (C/S = 2.00 +/- 1.00). Ten (10) samples were not spiked. The blood was aliquoted into serum collection tubes, centrifuged and the serum was separated from the cells. One (1) mL aliquots of each serum sample were placed at -20 °C, 2-8 °C, 21 °C and 30 °C. All samples were tested fresh and at defined time intervals during storage. Each sample was analyzed at n=3 with three Master lots of reagents using 3 instruments. The mean value for each storage condition and the overall mean across all storage conditions was calculated from the three determinations. The SD and CV(%) for anti-HBe-spiked samples were also calculated. Assay results < 1.00 C/S are classified negative. Assay results ≥ 1.00 C/S are classified reactive. For each storage condition, % differences were calculated from the fresh condition for anti-HBe-spiked samples using the following equation:

% Difference = Test Condition - Baseline Condition x 100 % Baseline Condition

The mean and range of the % differences, across all anti-HBe-spiked samples, for each condition were calculated.

The acceptance criteria were that no sample should be misclassified (i.e. gives a negative result rather than a reactive result) on any of the occasions on which they are determined. The acceptance criterion for the reactive samples (C/S ≥1.00) is that no sample should be

misclassified (i.e. gives a negative result rather than a reactive result) on any of the occasions on which they are determined. Maximum differences (change) in a mean result of + 50% and -33% would not cause a change in classification across the retest zone of 0.80 to 1.20 (C/S), therefore all % differences must fall within this range. For positive samples, no negative results were observed and for negative samples, no positive results were observed, for the mean results for any storage condition.
The studies supported stability of serum samples for 5 days at 2-8°C, for 4 weeks at -20°C and 10 hours at room temperature (up to 30 °C).

The effect of multiple freeze-thaw cycles on the stability of the anti-HBe in serum was also evaluated. Two serum samples reactive for anti-HBe were thawed and divided into six separate aliquots. One aliquot was stored at 2-8 °C and was considered the initial test sample while the other five were re-frozen at –20°C and subjected to five freeze/thaw cycles. Each aliquot was tested in duplicate, using two Master lots of reagents on two instruments. Same acceptance criteria, as described above, were applied. The data showed that up to five (5) freeze thaw cycles had no effect on the results of the VITROS Anti-HBe assay.

G. Reagent Stability

1. VITROS Anti-HBe Reagent Pack and Calibrator

   a. Long Term Stability (Shelf Life)

      VITROS Anti-HBe Reagent Packs and Calibrators that were subjected to a period of simulated transport by storage at 20°C for 2 days, and then returned to 2-8°C, were tested at monthly intervals up to 56 weeks using in-house controls and VITROS Anti-HBe Controls. Four runs were performed on 3 Master Lots of the VITROS Anti-HBe Reagent Pack and Calibrator at each timepoint. Each run contained duplicate determinations of the VITROS Anti-HBe Calibrator and singleton determinations of the QC In-house Controls (four levels) and the VITROS Anti-HBe Controls. The final shelf-life was defined as the time point where all parameters for all Master Lots pass the acceptability limits prior to the time points at which any same parameter for a Master Lot has failed the acceptance limits on two successive occasions.

      In addition, at initial, interim and expiry time points, runs were performed using a performance panel obtained from Boston Biomedica Inc. to assess the seroconversion sensitivity of the VITROS Anti-HBe assay throughout the shelf life. The results indicated that the performance panel samples retained their classification at the interim and expiry time points for all lots of material evaluated up to 52 weeks.

      The data supported the claimed shelf life stability of 40 weeks for the VITROS Anti-HBe Reagent Pack and Calibrator.

b. Temperature Stressing at -20 °C

VITROS Anti-HBe Reagent Packs and Calibrator were evaluated for effects of extreme temperature variation on their performance by exposing the reagents to freezing. All the reagents tested were subjected to a period of simulated transport (stored at 20°C for 2 days and then returned to 2-8°), prior to initiating the stability study, to mimic the effects of shipment. To evaluate the effect of freezing, three Master Lots of the VITROS Anti-HBe Reagent Packs and Calibrator were subjected to two freeze/thaw cycles and the performance was compared with Reagent Packs and Calibrators stored at 2-8 °C (unstressed). There were four runs performed for each combination of Reagent Packs and Calibrators. Each run consisted of duplicate determinations of the Calibrator and single determinations of the in-house Controls and the VITROS Anti-HBe Controls as follows:

Unstressed Reagent Pack and Unstressed Calibrator
Unstressed Reagent Pack and Stressed Calibrator
Stressed Reagent Pack and Unstressed Calibrator
Stressed Reagent Pack and Stressed Calibrator

The study results demonstrated that inadvertent freezing of the Reagent Pack and/or Calibrator had no adverse effect on Calibration Quality Parameters or Control results. It is recommended that the VITROS Anti-HBe Reagent Pack and Calibrator are not frozen.

c. Temperature Stressing at 30°C and 37°C

VITROS Anti-HBe Reagent Packs and Calibrator were evaluated for effects of extreme temperature variation on their performance by exposing the reagents to 30°C and 37°C. All the reagents tested were subjected to a period of simulated transport, prior to initiating the stability study, to mimic the effects of shipment. VITROS Anti-HBe Reagent Packs and Calibrators from three Master Lots were stored for 5 days at 30 °C (86 °F) or for 1 day at 37 °C (98.6 °F).

Four runs were performed for each combination of Reagent Packs and Calibrators. Each run consisted of duplicate determinations of the Calibrator and single determinations of the in-house Controls and the VITROS Anti-HBe Controls as follows:

Unstressed Reagent Pack and Unstressed Calibrator
Unstressed Reagent Pack and Stressed Calibrator
Stressed Reagent Pack and Unstressed Calibrator
Stressed Reagent Pack and Stressed Calibrator

The results showed that exposing the VITROS Anti-HBe Reagent Packs to a temperature of 30 °C (86 °F) for 5 days or 37 °C (98.6 °F) for 1 day caused detrimental effects on Calibrator Signal Index results. Inadvertent exposure of the Reagent Packs to these temperatures for the times stated would significantly compromise the performance of the VITROS Anti-HBe assay. However, inadvertent storage of the VITROS Anti-HBe Calibrator at 30°C for 5 days or 37°C for 1 day would not significantly compromise the performance of the VITROS Anti-HBe assay.

d. On Board Stability (Open Stability)

A real-time open Reagent Pack stability study was conducted using 3 Master Lots of Reagent Pack and Calibrator and aged generic reagents (Signal Reagent, Universal Wash Reagent) to assess the effect of open on board storage of the VITROS Anti-HBe Reagent Pack for a period of 12 weeks. All the reagents tested were subjected to a period of simulated transport (stored at 20°C for 2 days and then returned to 2-8°C prior to testing), prior to initiating the stability study, to mimic the effects of shipment. The VITROS Anti-HBe Reagent Packs and Calibrators that were subjected to a period of simulated transport, were opened and placed in an environmental chamber (4-8 °C at ≤40% relative humidity) for a period of up to 12 weeks to simulate the storage of the reagent packs on board the VITROS ECi/ECiQ System. Reagent packs and calibrators were removed from the chamber at 2 week intervals and used to test in-house controls and VITROS Anti-HBe Controls, performing four runs for each Master Lot. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls.

The data demonstrated that VITROS Anti-HBe Reagent Packs and Calibrators can be stored open, on board the VITROS ECi/ECiQ System for a period of up to 12 weeks.

e. Calibrator Open Stability

VITROS Anti-HBe Calibrators were reconstituted, pooled, transferred to sample cups and then stored at 2-8 °C and -20 °C for up to 13 weeks. The stored Calibrators were compared against fresh Calibrators at various time points throughout the 13 weeks using three Master Lots. The results at each time point indicated that there were no differences between the fresh and stored Calibrators, and no trends throughout the duration of storage were evident.

The data supports the storage of the VITROS Anti-HBe Calibrators, after reconstitution, for a period of up to 13 weeks at 2-8 °C, or up to 13 weeks at -20°C.

2. VITROS Anti-HBe Controls Stability

a. Long Term Stability (Shelf Life)

VITROS Anti-HBe Controls stability study was conducted using two lots of VITROS Anti-HBe Controls, three Master Lots of assay reagents (Reagent Pack/Calibrator) and aged generic reagents (Signal Reagent and Universal Wash Reagent). VITROS Anti-HBe Reagent Packs, Calibrators and Controls that were subjected to a period of simulated transport by storage at 20°C for 2 days, and then returned to 2-8°C, were tested at monthly intervals up to 56 weeks. Prior to the commencement of the stability study, results obtained from transported materials were compared to results obtained from non-transported materials to verify that QC In-house Controls results were not affected; thereby confirming that assay performance is maintained. Four runs were performed on 3 Master Lots of the VITROS Anti-HBe Reagent Pack and Calibrator at each timepoint. Each run contained duplicate determinations of the VITROS Anti-HBe Calibrator and singleton determinations of the QC In-house Controls (four levels) and the VITROS Anti-HBe Controls.

The study data support a shelf life stability for lyophilized VITROS Anti-HBe Controls of 52 weeks when stored at 2-8°C.

b. Reconstituted stability

Two lots of the VITROS Anti-HBe Controls were evaluated for stability after reconstitution of the lyophilized material. The testing was done with three Master Lots of the Reagent Pack and Calibrator) and aged generic reagents (Signal Reagent and Universal Wash Reagent). The Reagent Packs, Calibrators and the VITROS Anti-HBe Controls were subjected to simulated transport conditions (stored at 20°C for 2 days and then returned to 2-8°C prior to the commencement of the stability study). The results obtained from transported materials were compared to results obtained from non-transported materials to verify that QC In-house Controls results were not affected; thereby confirming that assay performance is maintained.

The VITROS Anti-HBe Controls were reconstituted, pooled and stored in sample cups at 2-8°C and at -20°C. The pooled Controls were tested on the day of reconstitution as time point 0. The VITROS Anti-HBe Controls stored at 2-8°C were subsequently tested on days 3, 4, 5 and 7. The VITROS Anti-HBe Controls stored at -20°C and thawed one time were tested on weeks 1, 2, 3 and 4 of the trial. Additionally, the VITROS Anti-HBe Controls stored at -20°C were subjected to 3 Freeze/Thaw cycles and were tested on week 4 of the trial. Four runs were performed at all time points using each Master Lot of Reagent Pack and Calibrator. Each run contained duplicate determinations of the Calibrator and

singleton determinations of the QC In-house Controls and VITROS Anti-HBe Controls.

All results met the predetermined acceptance criteria. The performance observed was comparable between the freshly reconstituted VITROS Anti-HBe Controls and the reconstituted VITROS Anti-HBe Controls stored at 2-8°C, -20°C and -20°C with 3 Freeze/Thaw cycles.

The data supports the stability claim of 5 days storage at 2-8°C or up to 4 weeks storage at -20°C with 3 Freeze/Thaw cycles for the VITROS Anti-HBe Controls after reconstitution.

3. Universal Wash Reagent

VITROS Anti-HBe Reagent Packs, Calibrators and Controls that were subjected to a period of simulated transport to mimic the effects of shipment were tested with three lots of VITROS Universal Wash Reagent at 0, 6 and 12 months of age to determine the effect of aged VITROS Universal Wash Reagent on VITROS Anti-HBe results.

The data indicated that the performance of the VITROS Anti-HBe assay is acceptable when used with VITROS Universal Wash Reagent which is either fresh, 6 or 12 months old.

4. Signal Reagent Stability

VITROS Anti-HBe Reagent Packs, Calibrator and Controls that were subjected to a period of simulated transport to mimic the effects of shipment were tested with four lots of VITROS Signal Reagent stored at 2-8 °C for up to 6 months to determine the effect of aged VITROS Signal Reagent on VITROS Anti-HBe results.

The data indicated that the performance of the VITROS Anti-HBe assay is acceptable when used with VITROS Signal Reagent which is either fresh, or 6 months old.

5. Preservative Effectiveness

Three aspects of microbiological control were studied in VITROS Anti-HBe Reagent Pack and Calibrator reagents:

- Determination of post-dispensing microbial load at 52 weeks
- Preservative concentration over a 52 week shelf-life
- Preservative efficacy 52 weeks post formulation

Results of the studies indicated that the level of the preservative used in the reagents was adequate for microbial control over the 52 week time period.

H. Seroconversion Sensitivity

The clinical sensitivity of the VITROS Anti-HBe assay was evaluated by testing six commercially available seroconversion panels. The VITROS Anti-HBe and FDA approved comparator anti-HBe test results are summarized below. The table presents the days elapsed from the date of the initial bleed for the last negative sample and first repeatedly reactive sample for the VITROS Anti-HBe and for the FDA approved comparator anti-HBe test as well as the difference between the two tests in identifying the first reactive panel member by number of days.

Days to Reactive anti-HBe Result					
	Comparator Anti-HBe Test		VITROS Anti-HBe Test		Difference in Days to Anti-HBe Reactive Result
Panel ID	-*	+**	-*	+**	**Comparator minus VITROS Anti-HBe Test**
PHM935B	61	75	103	118	-43
RP009	81	88	81	88	0
RP016	59	73	73	78	-5
RP017	78	140	78	140	0
6510	56	70	56	70	0
6513	98	112	98	112	0

* Post bleed day of last negative result, usually denotes previous bleed from first positive/reactive result.
**Post bleed day of first positive/reactive result.

I. Calibration Interval

The performance of the VITROS Anti-HBe assay within and beyond one calibration interval was evaluated in conjunction with the precision study (see below) which was conducted within one calibration interval (28 days). The study utilized a six member panel with C/S values around the cutoff, ranging from 0.40 to 1.56 C/S. Additional testing was conducted on days 29 and 30 to show that the analyzer would still yield valid results beyond the end of the 28 day cycle. The least squares regression analyses were performed within site and across sites.

The VITROS Anti-HBe assay demonstrated adequate performance throughout the calibration interval (28 days) and continued to perform successfully two days beyond the expiration of calibration.

J. Precision

The precision study was performed at three clinical testing sites over 28 days, using one lot of reagents. The mean C/S for the six panel members ranged from 0.38 to 1.60 for the total of 120 observations (3 sites). Each testing day included one replicate of Control 1 and Control 2, followed by two replicates of each panel member. A total of 40 observations were generated at each site for each panel member. The mean of the C/S ratio, relative variance, SD and %CV were calculated and presented for each site separately and overall. The data presented are a representation of the product performance, and were rounded following all calculations. The results are shown below.

Site	No. of Obs.	No. of Days	Mean VITROS Anti-HBe Test C/S Ratio	Precision					
				Within Day*		Between Days**		Total***	
				SD	CV (%)	SD	CV (%)	SD	CV (%)
Site 1	40	20	1.54	0.038	2.5	0.092	6.0	0.100	6.5
	40	20	1.41	0.043	3.1	0.070	5.0	0.083	5.9
	40	20	1.27	0.041	3.2	0.075	5.9	0.085	6.7
	40	20	0.80	0.021	2.6	0.036	4.5	0.042	5.2
	40	20	0.63	0.012	1.8	0.031	4.9	0.033	5.2
	40	20	0.40	0.007	1.6	0.018	4.6	0.020	4.9
Site 2	40	20	1.52	0.043	2.9	0.111	7.3	0.119	7.9
	40	20	1.39	0.040	2.8	0.100	7.2	0.107	7.7
	40	20	1.22	0.045	3.7	0.090	7.4	0.101	8.3
	40	20	0.77	0.025	3.3	0.044	5.7	0.051	6.6
	40	20	0.61	0.013	2.2	0.039	6.4	0.041	6.7
	40	20	0.38	0.008	2.0	0.024	6.2	0.025	6.5
Site 3	40	20	1.60	0.060	3.7	0.125	7.8	0.138	8.6
	40	20	1.49	0.041	2.8	0.147	9.9	0.153	10.2
	40	20	1.31	0.031	2.4	0.100	7.6	0.104	8.0
	40	20	0.84	0.017	2.1	0.065	7.8	0.067	8.0
	40	20	0.65	0.015	2.4	0.046	7.1	0.048	7.4
	40	20	0.41	0.006	1.4	0.030	7.2	0.030	7.4

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

** Between Days: Variability of the assay performance from day to day.

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

K. Reproducibility

Reproducibility of the assay was also evaluated incorporating between site and between lot variations. The study was performed at three external sites using three reagent lots to test three replicates each of a six member panel 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 are a representation of the product performance, and were rounded following all calculations.

Mean VITROS Anti-HBe Assay	Between Site *		Between Lot **		Total ***		No. of
C/S Ratio	SD	CV (%)	SD	CV (%)	SD	CV (%)	Obs.
1.41	0.098	7.0	0.079	5.6	0.185	13.2	162
1.32	0.087	6.6	0.057	4.3	0.160	12.1	162
1.18	0.067	5.7	0.054	4.5	0.141	11.9	162
0.73	0.051	6.9	0.031	4.2	0.083	11.3	162
0.57	0.047	8.2	0.023	4.0	0.067	11.8	162
0.36	0.031	8.5	0.013	3.6	0.043	11.9	162

*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.

L. Carryover Studies

Potential sources of cross-contamination on the VITROS ECi/ECiQ Immunodiagnostic System have been identified to be: 1) Sample-to-sample; 2) Reagent-to-reagent; and 3) Well wash-to-well wash. Sample-to-sample cross-contamination is mitigated by the use of disposable sample metering tips for each sample. The potential of reagent cross-contamination is of concern in particular between Anti-HBe and HBeAg assays, if run concurrently on the analyzer, since both assays utilize a labeled anti-HBe antibody as one of the critical reagents. The system was challenged in two experiments designed to detect a possible carryover of reagents.

The first study utilized 100 anti-HBe negative samples concurrently analyzed with the VITROS anti-HBe and the VITROS HBeAg assays (worst case scenario), to challenge both the reagent-to-sample and reagent-to-reagent carryover effect. The data showed no drifting or spiking in the negative result for anti-HBe which demonstrated that there is no reagent-to-sample or reagent-to-reagent carryover effect from the VITROS HBeAg assay reagents.

A second carryover challenge was conducted using 5 high anti-HBe positive samples as a worst case of potential sample carryover from well wash-to-well wash. A single negative sample was run at n=10 to establish a baseline. Next, the negative sample was placed in the tray immediately following each positive sample in the tray and the samples were processed in singleton. The experiment was repeated using a high negative sample that was created by diluting a positive sample to ~0.8 C/S. The results showed that there was no statistically significant difference in the mean of the negative sample. The mean of the negative sample results was 0.16 C/S (negative only) and 0.15 C/S (following high positive); the mean of the high negative was 0.79 C/S (negative only) and 0.77 C/S (following high positive).

The results demonstrated that there is no detectable cross-contamination that could affect the VITROS anti-HBe assay results when used on the VITROS ECi/ECiQ Immunodiagnostic System.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS Anti-HBe test among individuals with signs or symptoms of hepatitis (i.e. fatigue, anorexia, malaise, nausea, jaundice, abdominal pain, dark urine, headache, vomiting, weight loss, hepatomegaly and elevated liver function tests) 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.

A. Study Population

The prospective study population was divided into two groups. Population 1 consisted of 1976 subjects prospectively enrolled at four geographically separate locations; subjects were enrolled in Miami, FL (53.5%), in Dallas, TX (14.3%), in Newark, NJ (6.2%), and in Chicago, IL (26.0%). The group was Caucasian (18.9%), African American (51.0%), Hispanic (23.8%), and Asian (2.5%) with the remaining 3.8% represented by other ethnic groups. The group was 53.8% male and 46.2% female and ranged in age from 5 to 89 years.

Population 2 consisted of 311 subjects living in an area of India with high prevalence of HBV infection. All patients in Population 2 presented with signs or symptoms of viral hepatitis. The mean age of the population was 39 years and the median age was 40 years. Approximately 87% of the study subjects were ≤50 years of age. The minimum age was 18 years and the maximum age was 90 years. The population was 27% female and 73% male.

Testing of the samples with the VITROS Anti-HBe test was performed at diagnostic laboratories located in Miami, FL, Port Jefferson, NY and St. Paul, MN.

All study samples were also tested with the FDA approved comparator anti-HBe assay at diagnostic laboratories located in Miami, FL, Los Angeles, CA and St. Paul, MN.

B. HBV Disease Classification

All patients were serologically characterized using a hepatitis marker profile consisting of previously FDA approved tests for the detection of HBsAg, HBeAg, anti-HBc Total, anti-HBc IgM, anti-HBe, and anti-HBs (quantitative). The FDA approved tests' procedures were adhered to during the clinical study. The following positive (+) / negative (-) patterns for the six HBV serological markers were used to assign an HBV disease classification of chronic, recovered, vaccinated and not previously infected with HBV:

HBsAg*, **	HBeAg	aHBc IgM	aHBc Total	aHBe***	aHBs*** ≥10 mIU/mL	HBV Disease Classification
+	+	-	+	+	-	Chronic
+	+	-	+	-	+	Chronic
+	+	-	+	-	-	Chronic
+	+	-	+	-	I	Chronic
+	-	-	+	+	+	Chronic
+	-	-	+	+	-	Chronic
+	-	-	+	+	I	Chronic
+	-	-	+	-	+	Chronic
+	-	-	+	-	-	Chronic
+	-	-	+	I	-	Chronic
-	-	-	+	-	+	Recovered
-	-	-	+	-	-	Recovered
-	-	-	+	-	I	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	I	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected with HBV

* Positive = HBsAg test positive or reactive and confirmed by neutralization.

** Negative = HBsAg test negative or not confirmed by neutralization.

*** I = Indeterminate result.

C. Clinical performace

The clinical study data were analyzed following the assignment of HBV disease classifications. The data analysis included only the individuals that had hepatitis marker profiles consistent with chronic infection, recovered, vaccinated and not previously infected with HBV.

1. Expected Results

Of the 1976 subjects in Population 1 who were tested in the VITROS Anti-HBe clinical study, 1648 samples were derived from individuals who were chronically infected, recovered, vaccinated and those not previously infected with HBV. All 1648 were either at risk for HBV due to lifestyle, behavior, occupation, or a known exposure event or had signs and symptoms of hepatitis. Subjects in this group were enrolled in Miami, FL (51.2%), in Dallas, TX (15.0%), in Newark, NJ (6.4%), and in Chicago, IL (27.4%). The group was Caucasian (20.2%), African American (48.7%), Hispanic (25.4%), and Asian

(2.2%) with the remaining 3.5% represented by other ethnic groups. The group was 52.7% male and 47.3% female and ranged in age from 5 to 89 years. The distribution of VITROS Anti-HBe reactive and non-reactive results among the chronically infected, recovered, vaccinated and those not previously infected with HBV is presented, stratified by age and gender, in the following table.

Expected Results for Study Subjects in Population 1 (N=1648)

		Reactive		Negative		
Age Range	Gender	N*	Percent**	N***	Percent†	Total§
≤ 15	Female	0	0.0	2	100	2
	Male	0	0.0	4	100	4
16-20	Female	2	7.1	26	92.9	28
	Male	0	0.0	19	100	19
21-30	Female	1	0.8	126	99.2	127
	Male	1	0.8	124	99.2	125
31-40	Female	1	0.6	165	99.4	166
	Male	9	4.8	177	95.2	186
41-50	Female	1	0.5	196	99.5	197
	Male	17	6.3	252	93.7	269
51-60	Female	3	1.9	158	98.1	161
	Male	11	5.8	178	94.2	189
61-70	Female	1	1.6	63	98.4	64
	Male	1	1.7	58	98.3	59
> 70	Female	0	0.0	33	100	33
	Male	1	5.9	16	94.1	17
Unknown	Female	0	0.0	1	100	1
	Male	0	0.0	1	100	1
Total		49	3.0	1599	97.0	1648

* The total number (N) of subjects in each age range/gender category with reactive VITROS Anti-HBe results.

** The total number (N) of subjects in each age range/gender category that are reactive expressed as a percentage (%) of all subjects in that category.

*** The total number (N) of subjects in each age range/gender category with negative VITROS Anti-HBe results.

† The total number (N) of subjects in each age range/gender category that are negative expressed as a percentage (%) of all subjects in that category.

§ The total number (N) of subjects in each age range/gender category.

All subjects enrolled in Population 2 (N=311) were from an area in India with a high prevalence of HBV infection and all presented with signs or symptoms of viral hepatitis. Of the 311 subjects, 208 were from individuals who were chronically infected, recovered, vaccinated and those not previously infected with HBV. The mean age of these patients was 39 years and the median age was 40 years. Approximately 87% were <50 years of age. The minimum age was 18 years and the maximum age was 90 years. The group was 32.2% female and 67.8% male. The VITROS Anti-HBe test was reactive in 62.5% (130/208) of the individuals in this group. The distribution of VITROS Anti-HBe reactive and non-reactive results among the chronically infected, recovered, vaccinated and those not previously infected with HBV is presented, stratified by age and gender, in the following table.

Expected Results for Study Subjects in Population 2 (N=208)

Age Range	Gender	Reactive		Negative		Total[§]
		N*	Percent**	N***	Percent[†]	
18–20	Female	2	40.0	3	60.0	5
	Male	2	25.0	6	75.0	8
21–30	Female	16	61.5	10	38.5	26
	Male	15	51.7	14	48.3	29
31–40	Female	15	60.0	10	40.0	25
	Male	22	53.7	19	46.3	41
41–50	Female	4	57.1	3	42.9	7
	Male	30	76.9	9	23.1	39
51–60	Female	2	66.7	1	33.3	3
	Male	15	88.2	2	11.8	17
61–70	Female	1	100	0	0.0	1
	Male	5	83.3	1	16.7	6
>70	Male	1	100	0	0.0	1
Total		130	62.5	78	37.5	208

* The total number (N) of subjects in each age range/gender category with reactive VITROS Anti-HBe results.

** The total number (N) of subjects in each age range/gender category that are reactive, expressed as a percentage (%) of all subjects in that category.

*** The total number (N) of subjects in each age range/gender category with negative VITROS Anti-HBe results.

† The total number (N) of subjects in each age range/gender category that are negative, expressed as a percentage (%) of all subjects in that category.

§ The total number (N) of subjects in each age range/gender category.

Expected results for the VITROS Anti-HBe test were also determined using prospective samples from a population of pediatric subjects in Florida (N=165). The group was 47.9% male and 52.1% female, and the subjects' ages ranged from 2 through 21 years. The expected results are presented in the following table.

Expected Results for Pediatric Subjects (N=165)

Age Range	Gender	Reactive		Negative		Total[§]
		N*	Percent**	N***	Percent[†]	
2–4	Female	0	0.0	13	100	13
	Male	0	0.0	11	100	11
5–8	Female	0	0.0	18	100	18
	Male	0	0.0	17	100	17
9–12	Female	0	0.0	17	100	17
	Male	0	0.0	20	100	20
13–16	Female	0	0.0	21	100	21
	Male	0	0.0	14	100	14
17–21	Female	0	0.0	17	100	17
	Male	0	0.0	17	100	17
Total		0	0.0	165	100	165

* The total number (N) of subjects in each age range/gender category with reactive VITROS Anti-HBe results.

** The total number (N) of subjects in each age range/gender category that are reactive, expressed as a percentage (%) of all subjects in that category.

*** The total number (N) of subjects in each age range/gender category with negative VITROS Anti-HBe results.

† The total number (N) of subjects in each age range/gender category that are negative, expressed as a percentage (%) of all subjects in that category.

§ The total number (N) of subjects in each age range/gender category.

2. Agreement with a Comparator Assay

The VITROS Anti-HBe assay performance was evaluated for positive and negative agreement with an FDA approved comparator anti-HBe assay.

a) The following table compares the VITROS Anti-HBe results with the FDA approved comparator anti-HBe test results stratified by HBV disease classification for the 1648 subjects in Population 1 who were classified as chronically infected, recovered, vaccinated or not previously infected with HBV.

Comparison of Anti-HBe Test Results by HBV Disease Classification in Population 1 (N=1648)

HBV Disease Classification	Comparator Anti-HBe Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS Anti-HBe Test Result		VITROS Anti-HBe Test Result		VITROS Anti-HBe Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Chronic	47	1	0	21*	0	1	70
Recovered	0	0	1	218	0	0	219
HBV Vaccine Response	0	0	0	314	0	0	314
Not Previously Infected with HBV	0	0	1	1044	0	0	1045
Overall	47	1	2	1597	0	1	1648

* Three samples were HBeAg negative.

b) The following table compares the VITROS Anti-HBe results with the FDA approved comparator anti-HBe test results stratified by HBV disease classification for the 208 subjects in Population 2 who were classified as chronically infected, recovered, vaccinated or not previously infected with HBV.

Comparison of Anti-HBe Test Results by HBV Disease Classification in Population 2 (N=208)

HBV Disease Classification	Comparator Anti-HBe Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS Anti-HBe Test Result		VITROS Anti-HBe Test Result		VITROS Anti-HBe Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Chronic	130	3	0	52*	0	0	185
Recovered	0	0	0	3	0	0	3
HBV Vaccine Response	0	0	0	3	0	0	3
Not Previously Infected with HBV	0	0	0	17	0	0	17
Overall	130	3	0	75	0	0	208

* One sample was HBeAg negative.

3. Percent Agreement

Positive and negative percent agreement between the VITROS Anti-HBe test and the FDA approved comparator anti-HBe test were calculated for subjects by HBV disease classification. Positive percent agreement with the comparator anti-HBe test was determined by dividing the number of reactive VITROS Anti-HBe results by the total number of subjects positive with the comparator anti-HBe test. Negative percent agreement with the comparator anti-HBe test was determined by dividing the number of negative VITROS Anti-HBe results by the total number of subjects negative with the comparator anti-HBe test.

a) Population 1

The following table shows positive and negative percent agreement between the VITROS Anti-HBe test and the FDA approved comparator anti-HBe test along with the 95% exact confidence intervals for Population 1.

Positive and Negative Percent Agreement by HBV Disease Classification in Population 1 (N=1648)

HBV Disease Classification†	Positive Percent Agreement % (N/Total)*	95% Exact Confidence Interval	Negative Percent Agreement % (N/Total)*	95% Exact Confidence Interval
Chronic	95.92% (47/49)	86.02–99.50%	100.0% (21/21)***	83.89–100.0%
Recovered	N/A (0/0)**	N/A	99.54% (218/219)	97.48–99.99%
HBV Vaccine Response	N/A (0/0)**	N/A	100.0% (314/314)	98.83–100.0%
Not Previously Infected with HBV	N/A (0/0)**	N/A	99.90% (1044/1045)	99.47–100.0%

* VITROS Anti-HBe negative/ comparator indeterminate results (N=1) were considered VITROS Anti-HBe false negative when calculating positive agreement.

** There were no subjects with this HBV disease classification whose comparator test results fell within this category.

*** Three samples were HBeAg negative.

† No standalone anti-HBe test is adequate to stage HBV disease.

The positive percent agreement in samples with a chronic HBV disease classification in Population 1 was 95.92%. One VITROS Anti-HBe negative sample that was "indeterminate" by the comparator test was considered VITROS Anti-HBe false negative when calculating positive agreement. The negative percent agreement was 100.0%.

b) Population 2

Positive and negative percent agreement between the VITROS Anti-HBe test and the comparator anti-HBe test were calculated for subjects in Population 2. The following table summarizes these calculations and provides the 95% exact confidence intervals for this group.

Positive and Negative Percent Agreement by HBV Disease Classification in Population 2 (N=208)

HBV Disease Classification†	Positive Percent Agreement % (N/Total)	95% Exact Confidence Interval	Negative Percent Agreement % (N/Total)	95% Exact Confidence Interval
Chronic	97.74% (130/133)	93.55–99.53%	100.0% (52/52)***	93.15–100.0%
Recovered	N/A (0/0)**	N/A	100.0% (3/3)	N/A*
HBV Vaccine Response	N/A (0/0)**	N/A	100.0% (3/3)	N/A*
Not Previously Infected with HBV	N/A (0/0)**	N/A	100.0% (17/17)	80.49–100.0%

* Confidence intervals calculated on small numbers are not meaningful.

** There were no subjects with this HBV disease classification whose comparator test results fell within this category.

*** One sample was HBeAg negative.

† No standalone anti-HBe test is adequate to stage HBV disease.

The positive percent agreement in samples with chronic HBV disease classification in Population 2 was 97.74%. The negative percent agreement was 100.0%. There were no indeterminate comparator test results for the samples in Population 2.

c) Clinically Documented Chronic HBV Infection

The performance of the VITROS Anti-HBe test was also evaluated with samples from individuals in Population 1 with clinically documented chronic HBV infection. An individual was considered to have clinically documented chronic HBV infection if any one of the following criteria was met:

- HBsAg, HBV DNA or HBeAg FDA approved test was positive at least 6 months prior to the current positive HBsAg sample
- Documented diagnosis of HBV infection at least 6 months prior to the current positive HBsAg sample
- Medical record indicates two positive FDA approved tests for HBsAg, HBV DNA or HBeAg at least 6 months apart

Based on the above definitions, 36 individuals from Population 1 were considered to have a chronic HBV infection.

An additional 40 chronic HBV samples meeting these criteria were prospectively collected in Moscow, Russia. The subjects were Caucasian and ranged in age from 21 to 77 years. They were 85% male and 15% female. All 40 samples were tested at the testing site in Miami, FL. Thirty-three (82.5%) were positive with the comparator anti-HBe test and 25 (62.5%) were reactive with the VITROS Anti-HBe test. There were no comparator test indeterminate results among the 40 samples.

The following table summarizes the positive and negative percent agreement of the VITROS Anti-HBe assay with the comparator anti-HBe test in samples from individuals in the U.S. and Russia with clinically documented chronic HBV infection.

Positive and Negative Percent Agreement in Individuals with Chronic HBV Infection (N=76)

Population	Positive Percent Agreement % (N/Total)	95% Exact Confidence Interval	Negative Percent Agreement % (N/Total)	95% Exact Confidence Interval
Individuals with Chronic HBV Infection from Population 1 (N=36)	95.65% (22*/23)	78.05–99.89%	100.0% (13/13)	75.29–100.0%
Individuals with Chronic HBV Infection from Russia (N=40)	75.76% (25/33)	57.74–88.91%	100.0% (7/7)	N/A**

*One VITROS Anti-HBe negative/ comparator indeterminate result was considered VITROS Anti-HBe false negative when calculating positive agreement.

** Confidence intervals calculated on small numbers are not meaningful

d) Pregnant Women

Prospectively collected serum samples from healthy, pregnant women at high risk for exposure to HBV were tested to assess the clinical performance of the assay. Of the 244 women enrolled, there were 229 subjects whose hepatitis marker profiles were consistent with chronically infected, recovered, vaccinated or previously not infected with HBV. Of the 229 prospectively collected samples, 74.7% were obtained in Florida and 25.3% were obtained in Texas. In the population, 18.3% of the pregnant women were in the first trimester, 40.2% were in the second trimester, and 41.5% were in the third trimester of pregnancy. The following table provides a breakdown of the study population.

Demographic Profiles of Pregnant Women (N=229)

Collection Site	Florida N (%)*	Texas N (%)*	Total N (%)**
Total	171 (74.7)	58 (25.3)	229 (100.0)
Age (Years)			
18-20	29 (17.0)	22 (37.9)	51 (22.3)
21–30	72 (42.1)	26 (44.8)	98 (42.8)
31–40	63 (36.8)	9 (15.5)	72 (31.4)
41–50	7 (4.1)	1 (1.7)	8 (3.5)
>50	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity			
Caucasian	4 (2.3)	9 (15.5)	13 (5.7)
African-American	42 (24.6)	22 (37.9)	64 (27.9)
Hispanic	107 (62.6)	23 (39.7)	130 (56.8)
Asian	1 (0.6)	0 (0.0)	1 (0.4)
Indian	1 (0.6)	3 (5.2)	4 (1.7)
Haitian	10 (5.8)	0 (0.0)	10 (4.4)
Other	4 (2.3)	0 (0.0)	4 (1.7)
Unknown	2 (1.2)	1 (1.7)	3 (1.3)
Trimester			
First	6 (3.5)	36 (62.1)	42 (18.3)
Second	78 (45.6)	14 (24.1)	92 (40.2)
Third	87 (50.9)	8 (13.8)	95 (41.5)

* The number (N) of subjects at each site, expressed as a percentage (%) of analyzed subjects at each site.

** The total number (N) of subjects in each category, expressed as a percentage (%) of enrolled subjects (N=229).

The following table compares the VITROS Anti-HBe test with the comparator anti-HBe test among the population of pregnant women by trimester. None of the samples had indeterminate results with the comparator anti-HBe test.

Comparison of Anti-HBe Test Results in Pregnant Women by Trimester (N=229)

VITROS Anti-HBe Test Result	First Trimester			Second Trimester			Third Trimester		
	Comparator Anti-HBe Test Result			Comparator Anti-HBe Test Result			Comparator Anti-HBe Test Result		
	+	-	Total	+	-	Total	+	-	Total
Reactive	0	0	0	1	0	1	0	0	0
Negative	0	42	42	0	91	91	0	95	95
Total	0	42	42	1	91	92	0	95	95

Positive and negative percent agreement between the VITROS Anti-HBe test and the comparator anti-HBe test were calculated for the pregnant women in this study group. The following table summarizes these calculations and provides the 95% exact confidence intervals, where appropriate.

Positive and Negative Percent Agreement by HBV Disease Classification among Pregnant Women (N=229)

HBV Disease Classification	Positive Percent Agreement % (N/Total)	95% Exact Confidence Interval	Negative Percent Agreement % (N/Total)	95% Exact Confidence Interval
Chronic	100.0% (1/1)	N/A*	100.0% (2/2)	N/A*
Recovered	N/A (0/0)**	N/A	100.0% (11/11)	71.51–100.0%
HBV Vaccine Response	N/A (0/0)**	N/A	100.0% (27/27)	87.23–100.0%
Not Previously Infected with HBV	N/A (0/0)**	N/A	100.0% (188/188)	98.06–100.0%

* Confidence intervals calculated on small numbers are not meaningful.

** There were no subjects with this HBV disease classification whose comparator test results fell within this category.

e) Clinical Performance in Pediatric Subjects

Performance of the VITROS Anti-HBe assay in pediatric serum was determined using prospective samples from a population of pediatric subjects in Florida (N=165). The group was 47.9% male and 52.1% female, and the subjects' ages ranged from 2 through 21 years.

The following table compares the VITROS Anti-HBe results with the comparator anti-HBe results for the pediatric subjects. There were no comparator indeterminate results among the pediatric subjects.

Comparison of Anti-HBe Test Results in Pediatric Subjects (N=165)

VITROS Anti-HBe Test Result	Comparator Anti-HBe Test Result			Total
	Positive N	Negative N	Indeterminate N	N
Reactive	0	0	0	0
Negative	0	165	0	165
Total	0	165	0	165

The following table summarizes the percent agreement between the VITROS Anti-HBe test and the comparator anti-HBe test for the pediatric population. The table provides the 95% exact confidence intervals.

Positive and Negative Percent Agreement in Pediatric Subjects (N=165)

Subjects	Positive Percent Agreement % (N/Total)	95% Exact Confidence Intervals	Negative Percent Agreement % (N/Total)	95% Exact Confidence Intervals
Pediatric	N/A (0/0)	N/A	100.0% (165/165)	97.79–100.0%

There was 100% concordance between the VITROS Anti-HBe test and the FDA approved comparator anti-HBe test. None of the 165 samples was reactive with either the VITROS Anti-HBe test or the comparator anti-HBe test. There were no indeterminate comparator test results for the pediatric population.

The VITROS Anti-HBe assay performance was also evaluated using spiked anti-HBe reactive pediatric samples. Thirty (30) individual pediatric samples, non-reactive for anti-HBe, were spiked with an anti-HBe positive patient sample to a target level of 2.00–4.00 C/S and compared to matched spikes of an adult pool derived from a base matrix (pooled, defibrinated adult plasma, clarified, dialyzed and filtered). Ten (33%) samples were from subjects 2 to 11 years old and 20 (67%) were from subjects 12 to 21 years old. Each sample was run in the VITROS Anti-HBe test in duplicate. Mean results from the 30 spiked sample pairs were used to calculate the percent difference between the pediatric and the adult pool spike.

Twenty-seven of the 30 spiked pediatric samples gave reactivity lower than the spiked adult pool (derived from base matrix) ranging from 0.3% to 35.8% lower (average difference was 10.1% lower). Three of the 30 spiked pediatric samples gave reactivity higher than the adult pool, ranging from 0.7% to 8.1% higher (average difference was 3.5% higher).

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The adverse effects of the device are based on data collected in a clinical study conducted to support PMA approval as described above. As a diagnostic test, the VITROS Anti-HBe 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.

There were no adverse effects of the device reported while the study was conducted.

B. Effectiveness Conclusions

- The sensitivity and specificity of the VITROS Anti-HBe assay was shown to be comparable with the current commercially available FDA approved anti-

HBe assay in patients who are chronically infected with hepatitis or who have recovered from hepatitis B infection.

- The comparison of the performance of the VITROS Anti-HBe test in patients with chronic HBV infection among the study subjects, demonstrated a > 95% positive and negative percent agreement with the FDA approved comparator anti-HBe test.
- The comparison of the performance of the VITROS Anti-HBe test in those patients among the study subjects who recovered from HBV infection, demonstrated a > 99% positive and negative percent agreement with the FDA approved comparator anti-HBe test.
- The comparison of the performance of the VITROS Anti-HBe test in patients who were never previously infected or have been vaccinated against HBV demonstrated a > 99% negative percent agreement with the FDA approved comparator anti-HBe test.
- The performance of the VITROS Anti-HBe test was shown to be acceptable in pregnant women.
- The performance of the VITROS Anti-HBe test was shown to be acceptable in serum from pediatric patients (2 to 21 years old).
- Studies have shown that the VITROS Anti-HBe test has no significant cross-reactivity with the potentially cross-reacting clinical subgroups.
- Seroconversion sensitivity of the VITROS Anti-HBe assay has been shown to be acceptable by testing six commercial seroconversion panels.
- The stability of the VITROS Anti-HBe Reagent Pack and Calibrator has been demonstrated for a period of up to 40 weeks when stored at 2-8 °C.
- The stability of the VITROS Anti-HBe Reagent Pack and Calibrator, when stored on-board of the analyzer, has been demonstrated for a period of up to 12 weeks.
- The calibration interval is stable for 28 days when using the same lot of reagents.
- The stability of the VITROS Anti-HBe Controls has been demonstrated for a period of 52 weeks when stored at 2-8 °C. Once reconstituted, the Controls are stable for 5 days at 2-8 °C.
- The demonstrated precision of the VITROS Anti-HBe assay is within the expected range of this type of device.

The results from both the non-clinical and clinical studies indicate that the VITROS Anti-HBe assay is safe and effective for the *in vitro* qualitative detection of antibodies to the hepatitis B e antigen (Anti-HBe) in human adult and pediatric serum.

C. Overall Conclusions

The data in this application support a reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The submitted clinical studies have shown that the VITROS Anti-HBe assay, when compared to FDA approved comparator, has a similar ability to detect the presence of anti-HBe antibodies in serum specimens from individuals with chronic hepatitis B, or those

recovered from HBV infection. The rate of false positivity and false negativity are within acceptable limits compared to the comparator assay. It has been shown that the device has no demonstrable cross-reactivity with antibodies found in patients with potentially cross-reacting medical conditions. Therefore, this device should benefit the physician in providing additional information about a patient's progression to seroconversion which is important in the management of HBV infection.

XIII. CDRH DECISION

FDA issued an approval order on July 20, 2011. The final conditions of approval are cited in the approval order.

The applicant's manufacturing facilities were inspected and found to be in compliance with the devices Quality System (QS) regulation (21 CFR 820) on June 24, 2011.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.