

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Hepatitis B Virus e Antigen (HBeAg) Assay
Hepatitis B Virus e Antigen (HBeAg) Assay Control

Device Trade Name: VITROS[®] Immunodiagnostic Products HBeAg Reagent
Pack, Calibrator and Controls

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

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P090028

Date of FDA Notice of Approval: May 11, 2011

Expedited: Not Applicable

II. INDICATIONS FOR USE

VITROS[®] Immunodiagnostic Products HBeAg Reagent Pack

For the *in vitro* qualitative detection of hepatitis B e antigen (HBeAg) in human adult and pediatric (2 to 21 years old) serum from individuals who have symptoms of hepatitis or who may be at risk for hepatitis B virus (HBV) infection using the VITROS[®] ECi/ECiQ Immunodiagnostic System.

Test results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection.

VITROS[®] Immunodiagnostic Products HBeAg Calibrator

For use in the calibration of the VITROS[®] ECi/ECiQ Immunodiagnostic Systems for the *in vitro* qualitative detection of hepatitis B e antigen (HBeAg) in human adult and pediatric (2 to 21 years old) serum from individuals who have symptoms of hepatitis or who may be at risk for hepatitis B virus (HBV) infection.

VITROS[®] Immunodiagnostic Products HBeAg Controls

For use in monitoring the performance of the VITROS[®] ECi/ECiQ Immunodiagnostic Systems when used for the *in vitro* qualitative detection of hepatitis B e antigen (HBeAg) in human adult and pediatric (2 to 21 years old) serum from individuals who have

symptoms of hepatitis or who may be at risk for hepatitis B virus (HBV) infection when using the VITROS® Immunodiagnostic Products HBeAg Reagent Pack.

III. CONTRAINDICATIONS

- This assay has not been FDA licensed for the screening of blood, plasma and tissue donors.
- Test performance characteristics have not been established in patients under the age of 2, or in populations of immunocompromised or immunosuppressed patients.
- The VITROS® HBeAg test should not be used to test cord blood and plasma samples.
- The VITROS® HBeAg test should not be used to test turbid or hemolysed specimens.
- The performance of the VITROS® Immunodiagnostic Products HBeAg Controls has not been established with any other HBeAg test.
- For *in vitro* diagnostic use only.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VITROS® Immunodiagnostic Products HBeAg Reagent Pack, Calibrator, and Controls labeling.

V. DEVICE DESCRIPTION

A. Kit Configuration and Components

1. The VITROS® HBeAg Reagent Pack is composed of 3 reagents:
 - i. 100 coated wells (streptavidin source, bacterial; binds ≥ 3 ng biotin/well)
 - ii. 6.6 mL assay reagent in buffer with mouse serum and antimicrobial agent
 - iii. 6.6 mL conjugate reagent (source, HRP-mouse monoclonal anti-HBe 0.3 $\mu\text{g/mL}$ and biotin-mouse monoclonal anti-HBe 5.0 $\mu\text{g/mL}$) in buffer with sheep and mouse serum and antimicrobial agent
2. The VITROS® HBeAg Calibrator contains 3 vials of VITROS® HBeAg Calibrator (freeze-dried; HBeAg positive human plasma in HBeAg and anti-HBe negative human plasma; 0.7 ± 0.3 Units*/mL with antimicrobial agent), reconstitution volume 1.0 mL

* Paul-Ehrlich-Institute Reference Serum

3. The VITROS[®] HBeAg Controls contains 2 reagents:

- 3 vials of VITROS[®] HBeAg Control 1 (negative) (freeze-dried, defibrinated and delipidized human plasma with antimicrobial agent), reconstitution volume 1.0 mL.
- 3 vials of VITROS[®] HBeAg Control 2 (HBeAg positive) (freeze-dried, defibrinated and delipidized human plasma with antimicrobial agent), reconstitution volume 1.0 mL.

B. Assay Principle

VITROS[®] HBeAg assay

The VITROS[®] HBeAg assay is performed using the VITROS[®] HBeAg Reagent Pack and the VITROS[®] HBeAg Calibrator on the VITROS[®] ECi/ECiQ Immunodiagnostic System. An immunometric technique is used which involves the simultaneous reaction of HBeAg in the sample with biotinylated mouse monoclonal anti-HBe antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBe antibody in the conjugate. The immune complex is captured by streptavidin on the 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 VITROS[®] Analyzer reads the light signals. The amount of HRP conjugate bound is directly proportional to the concentration of HBeAg present in the sample.

VITROS[®] HBeAg Controls

The VITROS[®] HBe Controls are used to monitor the performance of the VITROS[®] HBeAg assay on the VITROS[®] Immunodiagnostic System and consists of two reagents. VITROS[®] HBeAg Control 1 contains freeze-dried, defibrinated and delipidized human plasma with antimicrobial agent, HBeAg and anti-HBe negative, whereas VITROS[®] HBeAg Control 2 is freeze-dried, defibrinated and delipidized human plasma with antimicrobial agent positive for HBeAg at a low concentration.

C. Calibration and Interpretation of Results

The VITROS[®] HBeAg assay utilizes a positive Reference Calibrator composed of inactivated human plasma, which is reactive for HBeAg. During assay development

when the light signal at the cut-off value was determined, the Reference Calibrator was included in the assay runs. Evaluation of accumulated data enabled expression of the cut-off signal, as a fraction of the Reference Calibrator signal. This fraction is used to maintain the position of the cut-off value from one calibration to another, compensating for variation in light signal between calibrations, across reagent lots and VITROS® ECI/ECiQ Analyzers. This fraction value was then confirmed by studies performed in Europe prior to the introduction of the assay into Europe and other regions of the world.

In order to maintain the position of the cut-off value in the end users' laboratory, a Reagent Lot specific Master Calibration (MC) that is traced to the original cut-off is provided to the customer in the lot calibration magnetic card that is supplied with the Product Calibrator. On receipt of a new Kit Lot, the customer scans information from the lot calibration magnetic card into the VITROS® Analyzer, transferring the MC to the Analyzer. The end user establishes a valid calibration for a specific Instrument/Lot by running the Product Calibrator in duplicate.

When routine specimens and quality control samples are run by the VITROS® Analyzer the sample signal is normalized relative to the cut-off signal according to the following equation:

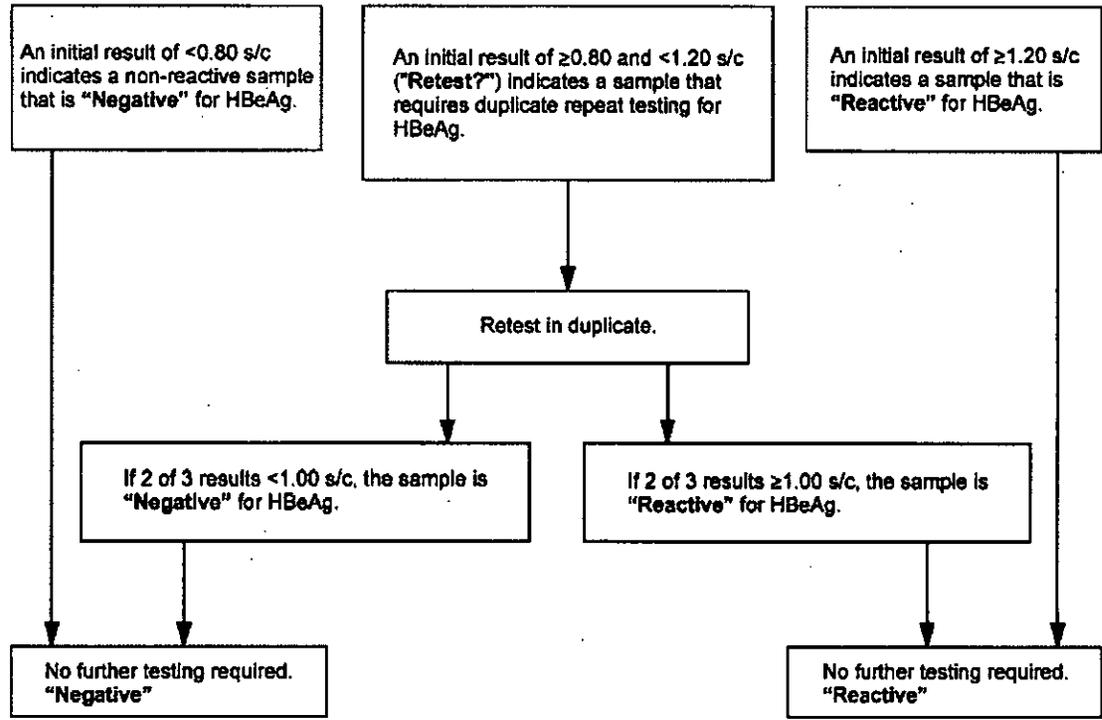
$$\text{Result} = \text{sample signal} / \text{cut-off signal}$$

Results are automatically calculated by the VITROS® ECI/ECiQ Immunodiagnostic Systems. Patient sample results are displayed with a "Negative", "Retest" or "Reactive" label. An initial result labeled with "Retest" indicates a sample that requires duplicate repeat testing for HBeAg.

VITROS® HBeAg Test Result (S/C)	<0.80	≥0.80 and <1.20	≥1.20
Result Text	Negative	Retest	Reactive

Final results are then manually interpreted using the algorithm below.

Testing Algorithm



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

VITROS® HBeAg Assay Result (S/C)	Conclusion from Testing Algorithm	Interpretation
0.80	Negative	Sample is non-reactive and presumed negative for HBeAg. This result should not be used alone, but in conjunction with other hepatitis B serological markers to determine disease state.
0.80 and <1.20	Retest in duplicate	Sample is non-reactive and presumed negative for HBeAg if 2 of 3 results are <1.00. Sample is reactive and presumed positive for HBeAg if 2 of 3 results are ≥1.00. These results should not be used alone, but in conjunction with other hepatitis B serological markers to determine disease state.
1.20	Reactive	Sample is reactive and presumed positive for HBeAg. This result should not be used alone, but in conjunction with other hepatitis B serological markers to determine disease state.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved *in vitro* diagnostic tests for serological markers of hepatitis B virus (HBV) infection which, when used in conjunction with a patient's medical history, clinical examination and other laboratory findings, can be used for diagnosis of HBV infection. In addition, there are currently two other FDA approved HBeAg devices.

VII. MARKETING HISTORY

The VITROS® Immunodiagnostic Products HBeAg 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	Kyrgyzstan
Armenia	Latvia
Australia	Lebanon
Austria	Liberia
Azerbaijan	Libya
Bangladesh	Lichtenstein
Belarus	Lithuania
Belgium	Luxembourg
Brazil	Malaysia
Brunei	Maldives
Bulgaria	Malta
Burma	Martinique
Canada	Moldova
Chile	Nepal
China	Netherlands
Colombia	New Zealand
Costa Rica	Nicaragua
Croatia	Nigeria
Cyprus	Norway
Czech Republic	Oman
Denmark	Panama
Dominican Republic	Paraguay
Ecuador	Peru
Egypt	Philippines
El Salvador	Poland
Estonia	Portugal
Finland	Reunion
France	Romania
French Antilles	Russia
French Guyana	Saudi Arabia
Georgia	Singapore
Germany	Slovak Republic
Greece	Slovenia
Guadeloupe	South Africa
Guatemala	Spain
Haiti	Sri Lanka
Honduras	Sweden
Hong Kong	Switzerland
Hungary	Taiwan
Iceland	Tajikistan
India	Thailand
Indonesia	Trinidad and Tobago
Iran	Turkey
Iraq	Turkmenistan
Ireland	United Arab Emirates
Israel	United Kingdom
Italy	Uruguay
Jamaica	Venezuela
Korea	Vietnam
Kuwait	

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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.

Failure of the test to perform as indicated or human error during performance of the test may lead to a false diagnosis and improper patient management.

The diagnosis of HBV infection requires the evaluation of the patient's blood for HBsAg, hepatitis B surface antibody (HBsAb), and hepatitis B core antibody (HBcAb). The HBeAg assay is not usually required in the initial diagnosis of HBV infection. However, it may be used as an aid in guiding antiviral therapy for patients that are chronically infected with HBV, in conjunction with HBV viral load test result. Seroconversion (i.e., conversion from HBeAg positive to HBeAg negative, followed by conversion from hepatitis B e antibody [anti-HBe] negative to anti-HBe positive) usually predicts long-term reduction in viral replication and may be used as a response marker to antiviral therapy. Therefore, repeatedly false negative or false positive results have the potential to lead to inappropriate treatment decisions.

A false negative HBeAg result may lead to premature withdrawal of antiviral therapy. A false reactive (false positive) result may lead to unnecessary prolonged antiviral therapy. Under these circumstances, there may be a safety concern for the patient. However, for chronic HBV patients who are on antiviral therapy, suppression of HBV DNA levels as measured by HBV viral load tests is often used as an end point of treatment.

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.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Antibody Characterization

The physio-chemical properties of the purified mouse monoclonal anti-HBe antibodies utilized in the VITROS[®] HBeAg assay were demonstrated by isotype determination, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), and isoelectric focusing (IEF) polyacrylamide gel electrophoresis. Investigation of the physio-chemical properties of the three lots of mouse monoclonal anti-HBe antibodies utilized by the VITROS[®] HBeAg assay determined that: 1) The isotype of the three lots of mouse monoclonal anti-HBe antibodies was IgG2a, kappa; 2) The SDS PAGE of the three lots of mouse monoclonal anti-HBe antibodies showed similar banding patterns with molecular weights typical of immunoglobulin heavy and light chains; 3) The IEF gel electrophoresis of the three lots of mouse

monoclonal anti-HBe antibodies showed similar banding patterns within part number, with pI values typical of monoclonal antibodies.

B. Cut-off Determination

During assay development the position of the cut-off signal was gathered experimentally based on an accumulation of clinical data. Light signals were measured on the VITROS[®] ECi/ECiQ Immunodiagnostic System for: 1) known HBeAg reactive patient samples; 2) seroconversion panels samples; 3) blood donor samples determined to be negative for HBeAg using other commercially available methods; 4) clinical samples from routine laboratory testing samples from patients with diseases clinically related to HBV infection but expected to be negative for HBeAg. The cut-off signal was then established as the light signal which gives the best discrimination between HBeAg reactive and HBeAg negative sample populations, to provide optimum specificity and sensitivity for the assay. The cut-off signal level was assigned a result of 1.00. Assay results ≥ 1.00 s/c indicate a reactive sample, positive for HBeAg. Assay results < 1.00 s/c indicate a non-reactive sample, negative for HBeAg.

The cut-off value established during assay development for the VITROS[®] HBeAg was then confirmed by studies performed in Europe prior to the introduction of the assay into Europe and other regions of the world. The European data demonstrated the following VITROS[®] HBeAg assay performance:

- Sensitivity of 100% (95% C.I., 98.00 to 100.00%) for HBeAg reactive samples (n=203)
- Seroconversion sensitivity equivalent to comparator assays (15 seroconversion panels and 1 mixed titer panel)
- Specificity of 99.88% (95% C.I., 99.57 to 99.99%) on blood donor samples (n=1676)
- Specificity of 99.84% (95% CI 99.14 – 100.00%) based on clinical samples. (n=645)
- Specificity of 100% (95% C.I., 95.80 to 100.00%) on clinical samples from potentially cross-reacting sub-groups (n=87)

C. Potentially Cross-Reacting Subgroups

To determine the analytical specificity of the VITROS® HBeAg assay with samples from potentially cross-reacting clinical subgroups, an analytical study testing a total of 200 patient samples representing various potentially cross-reacting clinical subgroups and 10 contrived samples simulating the *E.coli* subgroup samples was conducted. The majority of the samples tested in this analytical specificity study were characterized based on the relevant antibody markers rather than the relevant antigen markers. Of these samples, 13 were found to be repeatedly reactive in the VITROS® HBeAg test. All 10 cord blood samples were repeatedly reactive in the VITROS® HBeAg test and negative in an FDA approved comparator test. (Note: It is recommended in the PI not to use cord blood samples in the VITROS® HBeAg assay “Samples Not Recommended” section under “Specimen Collection, Preparation, and Storage”). Two (2) of the 3 remaining repeatedly reactive samples were also positive in the FDA approved comparator test. One (1) sample from the Rheumatoid Factor sub-group was reactive in the VITROS® HBeAg test and negative in the FDA approved comparator test. The comparison of the analytical specificity between the two assays is summarized in the following table:

Sample Category	No. Of Samples Tested	No. Negative	No. Initial Reactive	No. Repeat Reactive	No. Positive FDA Approved Comparator test*
Hepatitis C Virus Infection (HCV)	10	10	0	0	0
Cytomegalovirus (CMV)	10	10	0	0	0
Epstein Barr Virus (EBV)	10	10	0	0	0
Herpes Simplex Virus (HSV)	10	10	0	0	0
Rubella	10	10	0	0	0
Syphilis	10	10	0	0	0
Toxoplasmosis	10	10	0	0	0
Elevated Liver Enzymes (ALT)	10	10	0	0	0
Rheumatoid Factor(RF)	10	9	1	1	0
Anti-Nuclear Antibodies(ANA)	10	10	0	0	0
Heterophilic anti-mouse antibodies (HAMA) Past	10	10	0	0	0
Hepatitis A Infection/Immunized (HAVTotal)	10	10	0	0	0
Non-viral Liver Disease	10	9	1	1	1
AutoImmune Disease (RA) Rheumatoid Arthritis	10	10	0	0	0
AutoImmune Disease (SLE)	10	9	1	1	1
Parvovirus B19 Infection	10	10	0	0	0
HIV 1/2	10	10	0	0	0
HTLV 1/2	10	10	0	0	0
Recent Influenza Vaccine Recipients	10	10	0	0	0
Cord Blood	10	0	10	10	0
Escherichia coli (10 ⁴ cfu/mL) (<i>E.Coli</i>)	10	10	0	0	0

* FDA approved test

Additionally, 107 samples from 10 viral antigen sub-group categories were also assayed with the VITROS[®] HBeAg assay. None of the samples were found to result in a reactive classification in the VITROS[®] HBeAg assay.

Viral Antigen Subgroup	No. Of Samples Tested	No. HBeAg Negative
Hepatitis A Antigen	10	10
Hepatitis C Virus (HCV)	10	10
Cytomegalovirus Grade 2 (CMV)	10	10
Herpes Simplex Virus Type 1 Grade 2 (HSV-1)	10	10
Herpes Simplex Virus Type 2 Grade 2 (HSV-2)	10	10
Rubella K0S Concentrate	10	10
Influenza A Grade 2	10	10
Influenza B Antigen	10	10
EBV P3H3 Cell Extract	10	10
HIV 1/2	17	17

D. Interfering Substances

The effect of levels of potential interferents (lipemia, hemoglobin, bilirubin, biotin, sodium azide and dipyrone) on the performance of the VITROS[®] HBeAg assay was determined using methods based on Clinical and Laboratory Standards Institute (CLSI) EP7-A2 and an in-house method for hemoglobin. All interfering substance evaluations were performed using patient samples. Performance was assessed in both negative and positive samples for the VITROS[®] HBeAg assay. Samples were tested in triplicate using two master lots on two VITROS[®] ECi/ECiQ Immunodiagnostic Systems. Negative patient samples were prepared by pooling fresh non-reactive human serum. Reactive patient samples were prepared by spiking a portion of the negative pool with HBeAg reactive serum to achieve the targeted value (s/c of 2.00±1.00). The potential interferents prepared at incremental levels were used to spike the positive and negative human serum samples to achieve the target levels of the potential interferents. The acceptance criteria for the determination of non-interference were that “no sample should be misclassified (i.e. gives a positive result rather than a negative result for the negative samples or gives a negative result rather than a positive result for the reactive samples) on any of the occasions on which they were tested”, and “differences in mean result of + 50% and – 33% would not cause a change in classification across the result borderline (retest) region of 0.80 to 1.20 (s/c).” The acceptance criteria were set at -33% to +50% changes in s/c based on a worst case scenario. These limits are set so that a shift of a sample result at the edges of the retest zone (0.80 to 1.20 s/c) would not change classification from negative to positive or positive to negative. A -33% shift of a sample at 1.20 s/c would drop the reported value to 0.80 s/c and a +50% shift of a sample at 0.80 s/c would raise the reported value to 1.20 s/c. Shifts of less than this magnitude would yield results in

the retest zone and provide additional opportunity to assess the true nature of the samples.

The results of the study demonstrated that bilirubin, triolein, biotin and dipyrone at the highest concentrations (0.35 mmol/L or 20 mg/dL, 339 mmol/L or 3 g/dL, 40.8 nmol/L or 1 ug/dL, and 3.0 mmol/L or 100 mg/dL, respectively) tested cause no misclassification of results, such that the samples that are reactive are always reactive and that the negative samples are always negative in the VITROS[®] HBeAg assay.

Sodium Azide at a concentration of 1.0 g/dL resulted in misclassification of a reactive to a negative result. Sodium Azide at a concentration of 1.0 g/dL (1.0%) resulted in a significant decrease in the s/c value of the reactive pool. Typical commercial controls that use sodium azide as a preservative contain 0.1% (0.1g/dL) which results in an approximately 13% decrease in results. (Note: A limitation is included in the PI, "Other Limitations" section under "Limitations of the Procedure").

Hemoglobin at a concentration of 125 mg/dL and above resulted in misclassification of negative samples. Increasing hemoglobin concentrations yielded increasing s/c values. At a hemoglobin concentration of 62 mg/dL a 50% increase in s/c is observed in negative samples and would thus meet the acceptance criteria. Tested hemoglobin concentrations greater than 62 mg/dL yielded increases in excess of 50% and could thus result in a misclassification. (Note: It is recommended in the PI not to use hemolysed samples in the VITROS[®] HBeAg assay "Samples Not Recommended" section under "Specimen Collection, Preparation, and Storage").

The effect of elevated serum protein levels on the VITROS[®] HBeAg test was not evaluated. (Note: A limitation is included in the PI, "Other Limitations" section under "Limitations of the Procedure", which states "Each clinical laboratory should verify the performance of this assay with samples with high protein content" based on CLSI EP7-A2 recommendations.)

E. Analytical Sensitivity

An analytical sensitivity study confirming the concentration at the cut-off of the VITROS[®] HBeAg assay using an 11 member dilution series of the Paul-Ehrlich Institute (PEI) HBeAg reference serum with HBeAg negative serum was carried out. A total of 6 replicates of each dilution were tested with 2 reagent lots on 2 VITROS[®] ECi/ECiQ Systems. The mean result of each dilution was determined using the results of all replicates combined. A linear regression was performed using a plot of the mean result from the VITROS[®] HBeAg assay as the "y" variable versus the calculated concentration of each dilution as the "x" variable, and was used to determine the HBeAg concentration at the cut-off (s/c = 1.00). With an estimated analytical sensitivity of 0.11 PEI Units/mL at the cut-off (s/c = 1.00), the minimum analytical sensitivity that can be achieved by an end user with assurance when factoring in variability across reagent lots, instruments, and occasions is 0.35 PEI Units/mL.

F. Carryover Study

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. Reagent-to-reagent cross-contamination is mitigated by the fact that the system specification for reagent carryover is <1 in 150,000 per event. Therefore the risks of reagent-to-well or reagent bottle-to-reagent bottle cross-contamination impacting assay results are minimum. Well wash-to-well wash cross-contamination is mitigated by the fact that the system specification for well wash carry over is 1 part per 2 million (1:2,000,000) of previous reaction mix into the subsequent reaction well. The risk of well wash-to-well wash cross-contamination impacting assay results is also minimum.

In an effort to independently challenge the effects of potential reagent cross-contamination, a study was conducted where a single negative sample was run on the VITROS® ECi/ECiQ Immunodiagnostic System. This sample was placed into multiple cups to simulate testing 100 separate patient samples with alternating VITROS® anti-HBe and VITROS® HBeAg assays. Each sample cup was programmed to run both assays simultaneously and the system was run continuously until both fresh packs of 100 tests were depleted. This testing showed no drifting or spiking in the negative result for HBeAg. This is a worse case study that would challenge both reagent-to-well and reagent bottle-to-reagent bottle carry-overs.

A second carryover challenge was conducted using 6 high positive samples (1,805-2,656 s/c) as a worst case of potential sample carryover from well wash-to-well wash. A single negative sample was run first (10 cups, 1 rep each) without any positive samples to establish a baseline. Next, the negative sample was placed immediately following each positive sample in the tray and the samples processed in singleton. Finally, another round of negative sample only was run at the end of the test. The results showed that there was no statistically significant difference in the mean of the negative sample. The means of the negative for all 3 runs was 0.23 s/c and the distributions were overlapping with no significant elevation of any negative samples following a high positive sample.

G. Stability Studies

1. Sample Stability

Analytical studies determining the effects of storage on serum samples at 2-8°C for up to 7 days, and at -20°C for up to 4 weeks, and the effects of room temperature (approximately 21°C) serum sample storage on results obtained with

the VITROS® HBeAg assay were carried out. Ten (10) HBeAg positive (spiked with HBeAg level just above the cut-off) and HBeAg negative serum samples were stored at -20°C, at 2-8°C and at 21°C. The time points tested were day 0 (unstressed), 5 days, and 7 days for storage stability at 2-8°C. The time points tested for storage stability at 21°C were hour 0 (unstressed), hour 8, and hour 10. The time points tested for storage stability at -20°C were day 0 (unstressed) and 4 weeks. The acceptance criteria for the determination of serum stability were no sample should be misclassified (i.e. gives a positive result rather than a negative result for the negative samples or gives a negative result rather than a positive result for the reactive samples) on any of the occasions on which they were determined, and the mean % differences must fall within + 50% and - 33%. (Note: These limits were set so that a shift of a sample result at the edges of the retest zone (0.80 to 1.20 s/c) would not change classification from negative to positive or positive to negative. A -33% shift of a sample at 1.20 s/c would drop the reported value to 0.80 s/c and a +50% shift of a sample at 0.80 s/c would raise the reported value to 1.20 s/c. Shifts of less than this magnitude would yield results in the retest zone and provide additional opportunity to assess the true nature of the samples.)

The study results indicated that serum samples are suitable for use in the VITROS® HBeAg assay and that their suitability is not affected by storage of up to 10 hours in room temperature (21°C), up to 7 days at 2-8°C and after 4 weeks at -20°C.

Analytical studies determining the effects of multiple freeze-thaw cycles on results for samples tested with the VITROS® HBeAg assay were also carried out. A total of two HBeAg reactive serum samples with 1.10 initial mean s/c and 1.50 initial mean s/c, respectively, were utilized in the study, no HBeAg negative serum sample was tested in the study. A total of 5 freeze/thaw cycles were performed. Time points tested for the 5 freeze/thaw cycles were time 0 (fresh) and after each freeze/thaw cycle. The acceptance criteria for the determination of no effects of freeze/thaw for the reactive samples are 1) 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; and 2) differences (change) in mean result within the range of + 50% and -33% . (Note: These limits were set so that a shift of a sample result at the edges of the retest zone (0.80 to 1.20 s/c) would not change classification from negative to positive or positive to negative. A -33% shift of a sample at 1.20 s/c would drop the reported value to 0.80 s/c and a +50% shift of a sample at 0.80 s/c would raise the reported value to 1.20 s/c. Shifts of less than this magnitude would yield results in the retest zone and provide additional opportunity to assess the true nature of the samples.)

The study results demonstrated that subjecting HBeAg reactive samples to as many as 5 freeze/thaw cycles does not affect results in the VITROS® HBeAg assay.

2. VITROS[®] HBeAg Reagent Pack and Calibrator Stability Studies

i. Real Time (Shelf Life) Stability of the VITROS[®] HBeAg Reagent Pack and Calibrator

A real-time closed Reagent Pack and Calibrator stability study was conducted using 3 Lots of Master Lot (Reagent Pack/Calibrator) and aged generic reagents (Signal Reagent, Universal Wash Reagent) to assess the stability of the VITROS[®] HBeAg Reagent Pack and Calibrator. Data from runs performed at weeks 0, 4, 9, 13, 16, 18, 20, 22, 26, 30, 36, 45, 52 and 56 using VITROS[®] HBeAg Reagent Pack and Calibrator stored at 2-8°C were used. The Reagent Packs and Calibrators were subjected to simulated transport conditions by storing at 20°C for 2 days and then returned to 2-8°C prior to testing. 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 at each time-point for each Lot. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls, including 1) Control C-A: negative for HBeAg (It is used to monitor specificity and must always give a clear negative result. It has an approximate value of 0.25 s/c); 2) Control C-B: weak HBeAg positive (It is used to track seroconversion sensitivity. It has an approximate value of 2.0 s/c); 3) Control C-C: HBeAg positive (It is used to monitor the dose response characteristics of the assay. It has an approximate value of 5.5 s/c); and 4) Control C-D: HBeAg positive (It is also used to monitor the dose response characteristics of the assay. It has an approximate value of 13.0 s/c.), and VITROS[®] HBeAg Controls, including 1) VITROS[®] HBeAg Control C1: negative for HBeAg; and 2) VITROS[®] HBeAg Control C2: HBeAg positive.

At week 0, week 26 and week 52 time points, runs were performed using a performance panel obtained from the Zeptometrix Corporation to assess the seroconversion sensitivity through the shelf life of the VITROS[®] HBeAg assay. The performance panel retained their classification at week 26 and week 52.

The study data showed that the stability trial performance of the VITROS[®] HBeAg Reagent Pack and Calibrator are acceptable up to the week 30 and week 36 time points when stored at 2-8°C. An upward, positive trend was observed between weeks 45 and 56 for the negative controls, QC In-house Control A3 and VITROS[®] HBeAg Control C1 in Master Lot 3. Therefore, the shelf life is restricted to 32 weeks, which is 4 weeks prior to the last successful time point (36 weeks time point).

Stability studies conducted on three lots of reagents demonstrate a shelf life stability of the VITROS[®] HBeAg Reagent Pack and Calibrator for 32 weeks when stored at 2-8°C.

ii. Temperature Stressing of the VITROS[®] HBeAg Reagent Pack and Calibrator at -20°C

An analytical study was conducted to determine the effect of temperature stressing the VITROS[®] HBeAg assay by freezing and thawing Reagent Packs and Calibrators. The Reagent Packs and Calibrators were subjected to simulated transport conditions before starting the stability trial. To simulate the transport conditions, Reagent Packs and Calibrators were stored at 20°C for 2 days and then returned to 2-8°C prior to stressing. Prior to the commencement of the stressing 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. VITROS[®] HBeAg Reagent Packs and Calibrators from 3 Master Lots were subjected to 2 freeze/thaw cycles at -20°C. The Reagent Packs and Calibrators were placed on their sides during these cycles. VITROS[®] HBeAg Reagent Packs and Calibrators stored on their sides at 2-8°C were used as unstressed controls.

Four runs were performed at each time-point for each Lot. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls as those described in detail in section G.2.i, using the following combinations of Reagents:

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

All results met the predetermined acceptance criteria.

The study results demonstrated that inadvertent freezing of the Reagent Pack and/or Calibrator would not significantly compromise the performance of the VITROS[®] HBeAg assay. (Note: However, as a precaution, it is still recommended in the PI to store VITROS[®] HBeAg Reagent Pack and Calibrator unopened at 2-8°C (“Calibrator Storage and Preparation” section and “Reagent Pack Storage and Preparation” section under “Reagents”))

iii. Temperature Stressing of the VITROS[®] HBeAg Reagent Pack and Calibrator at 30°C and at 37°C

An analytical study was conducted to determine the effect of temperature stressing the VITROS[®] HBeAg assay by storing the Reagent Packs and Calibrators for 5 days at 30°C or 1 day at 37°C. The Reagent Packs and Calibrators were subjected to simulated transport conditions before starting the stability trial. To simulate the transport conditions, Reagent Packs and Calibrators were stored at 20°C for 2 days and then returned to 2-8°C prior to testing or stressing. Prior to the commencement of the stressing study, results obtained from transported materials were compared to results obtained from non-transported materials to verify that QC In-house Control results were not affected; thereby confirming that assay performance is maintained. VITROS[®] HBeAg Reagent Packs and Calibrators from 3 Master Lots were stored at either 30°C for 5 days or 37°C for 1 day. The Reagent Packs and Calibrators were placed on their sides during these periods. VITROS[®] HBeAg assay Reagent Packs and Calibrators stored on their sides at 2-8°C were used as unstressed controls.

Four runs were performed at each time-point for each Lot. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls as those described in detail in section G.2.i, using the following combinations of Reagents:

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

All results met the predetermined acceptance criteria.

The study results demonstrated that inadvertent storage at 30°C for up to 5 days or 37°C for up to 1 day of the Reagent Pack or Calibrator would not significantly compromise the performance of the VITROS[®] HBeAg assay. (Note: However, as a precaution, it is still recommended in the PI to store VITROS[®] HBeAg Reagent Pack and Calibrator unopened at 2-8°C (“Calibrator Storage and Preparation” section and “Reagent Pack Storage and Preparation” section under “Reagents”))

iv. On-Board Stability – Open VITROS[®] HBeAg Reagent Pack

A real-time open Reagent Pack stability study was conducted using 3 Lots of Master Lot (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[®] HBeAg Reagent Pack for a period of 12 weeks. The Reagent Packs and Calibrators were subjected to a period of simulated transport. To simulate the transport conditions, Reagent Packs and Calibrators were stored at 20°C for 2 days and then returned to 2-8°C prior to testing.

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.

Transported Reagent Packs from 3 Master Lots were opened and placed in an Environmental Chamber (4-8°C at 40% relative humidity) for a period of 12 weeks to simulate the storage of the Reagent Packs on board the VITROS® Analyzer. These Reagent Packs were tested at weeks 2, 4, 5, 6, 7, 8 and 12 performing four runs for each Master Lot of transported material. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls, as those described in detail in section G.2.i. Fresh Reagent Packs stored at 2-8°C from each of the 3 Master Lots were tested in the same manner at weeks 0 and 12 as unstressed controls.

All results met the predetermined acceptance criteria. Percentage differences between open stored and freshly opened Reagent Packs for the QC In-house Controls were calculated on QC In-house Controls C-B, C-C and C-D, and HBeAg Control C2. The overall % difference across the 3 Master Lots is < 10%, with all results within the predetermined acceptance criteria, there were no significant trends observed.

The study data supports the onboard storage of the VITROS® HBeAg Reagent Pack for a period up to 12 weeks with typical Reagent Pack use for the VITROS® HBeAg assay.

v. “Off-Board” Stability – Open VITROS® HBeAg Calibrator

A real-time stability study was carried out using 3 Lots of Master Lot (Reagent Pack and Calibrator) and aged generic reagents (Signal Reagent, Universal Wash Reagent) to assess the effect of open “off-board” storage at 2-8°C and -20°C of the Calibrator on the performance of the VITROS® HBeAg assay. The Reagent Packs and Calibrators were subjected to simulated transport conditions. To simulate the transport conditions, Reagent Packs and Calibrators were stored at 20°C for 2 days and then returned to 2-8°C prior to testing. 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.

The Calibrators were opened, reconstituted, pooled and stored in sample cups at 2-8°C and at -20°C. The pooled Calibrators were tested at the initial time point. The Calibrators stored at 2-8°C and -20°C were tested at weeks 5, 10 and 13. Four runs were performed at each time point for each Master Lot. Each run contained duplicate determinations of the fresh reconstituted and

pooled opened stored Calibrators (2-8°C and -20°C) and single determinations of the QC In-house Controls as those described in detail in section G.2.i.

All results met the predetermined acceptance criteria. No obvious and significant trends up to 5 weeks at 2-8°C and 13 weeks at -20°C were observed based on the review of the graphical representations of the VITROS® HBeAg Calibrator Open “Off-board” Storage Stability Study data.

The data supports the stability claim of the storage of the VITROS® HBeAg Calibrator, after opening, for a period of up to 5 weeks at 2-8°C, and up to 13 weeks at -20°C.

3. VITROS® HBeAg Controls Stability Studies

i. Real Time (Shelf Life) Stability of the VITROS® HBeAg Controls

A real-time closed (un-reconstituted) VITROS® HBeAg Controls study was conducted using 2 lots of VITROS® HBeAg Controls, 3 Lots of Master Lot (Reagent Pack/Calibrator) and aged generic reagents (Signal Reagent, Universal Wash Reagent) to assess the stability of the VITROS® HBeAg Controls. Data from runs performed at weeks 0, 4, 9, 13, 16, 18, 20, 22, 26, 30, 36, 45, 52 and 56 using VITROS® HBeAg Reagent Pack and Calibrator and VITROS® HBeAg Controls stored at 2-8°C were used. The Reagent Packs and Calibrators were subjected to simulated transport conditions by storing at 20°C for 2 days and then returned to 2-8°C prior to testing. 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 at each time-point for each Lot. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls, as those described in detail in section G.2.i.

The study data showed that the stability trial performance of the VITROS® HBeAg Controls are acceptable up to the week 52 and week 56 time points when stored at 2-8°C.

Stability studies demonstrate a shelf life stability of the VITROS® HBeAg Controls for 52 weeks when stored at 2-8°C.

ii. Stability of the reconstituted VITROS® HBeAg Controls

Analytical studies were carried out using 3 Lots of Master Lot (Reagent Pack and Calibrator) and aged generic reagents (Signal Reagent, Universal Wash

Reagent) and 2 Lots of the HBeAg Controls to assess the effect of storage under various conditions on the results obtained for the VITROS[®] HBeAg Controls with the VITROS[®] HBeAg assay. The Reagent Packs, Calibrators and VITROS[®] HBeAg Controls were subjected to simulated transport conditions. To simulate the transport conditions, Reagent Packs, Calibrators and VITROS[®] HBe Controls were stored at 20°C for 2 days and then returned to 2-8°C prior to testing. 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.

The VITROS[®] HBeAg Controls were reconstituted, pooled and stored in sample cups at 2-8°C and at -20°C. The pooled VITROS[®] HBe Controls were tested at the initial time point. The VITROS[®] HBeAg Controls stored at 2-8°C were tested on days 0, 3, 4, 5 and 7. The VITROS[®] HBeAg Controls stored at -20°C with one Freeze/Thaw cycle were tested on weeks 0, 1, 2, 3 and 4. The VITROS[®] HBeAg Controls stored at -20°C with 3 Freeze/Thaw cycles were tested on week 4 only. Four runs were performed at all time points using each Master Lot. Each run contained duplicate determinations of the Calibrator and singleton determinations of the QC In-house Controls, as those described in detail in section G.2.i.

All results met the predetermined acceptance criteria. The performance observed was comparable between freshly reconstituted VITROS[®] HBeAg Controls and reconstituted VITROS[®] HBeAg Controls stored at 2-8°C, -20°C and -20°C with 3 Freeze/Thaw cycles. No obvious and significant trends up to 5 days storage at 2-8°C or up to 4 weeks storage at -20°C with 3 Freeze/Thaw cycles for the VITROS[®] HBeAg Controls were observed based on the review of the graphical representations of the VITROS[®] HBeAg Controls Storage Stability Study data.

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[®] HBeAg Controls.

4. VITROS[®] Universal Wash Reagent Stability Study

An analytical study was conducted using 1 Lot of the Reagent Pack and Calibrator and 3 Lots of the VITROS[®] Universal Wash Reagent to assess the performance of the VITROS[®] HBeAg assay when tested with fresh VITROS[®] Universal Wash Reagent and VITROS[®] Universal Wash Reagent that has been stored for 6 and 12 months at 2-8°C. Reagent Packs, Calibrators and VITROS[®] HBeAg Controls were subjected to a period of simulated transport. This was to mimic the effects of the possible conditions that may occur during transport to customers. To simulate the transport conditions, Reagent Packs, Calibrators and

VITROS[®] HBeAg Controls were stored at 20°C for 2 days and then returned to 2-8°C prior to testing in the VITROS[®] Universal Wash Reagent Study. Prior to the commencement of the 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.

Using 1 Master Lot of transported materials, four runs were performed at month 0, 6 and 12 using 3 lots of VITROS[®] Universal Wash Reagent. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls as those described in detail in section G.2.i,

All results met the predetermined acceptance criteria. No obvious and significant performance difference was observed between results generated using VITROS[®] Universal Wash Reagent that is 6 and 12 months old (stored at 2-8°C) and fresh VITROS[®] Universal Wash Reagent based on the review of the line data of the VITROS[®] Universal Wash Reagent Stability Study.

The study data shows that the performance of the VITROS[®] HBeAg Reagent Pack and Calibrator is acceptable when tested with fresh VITROS[®] Universal Wash Reagent and VITROS[®] Universal Wash Reagent that is stored at 2-8°C for up to 12 months.

5. VITROS[®] Signal Reagent Stability Study

An analytical study was conducted using 1 Lot of the Reagent Pack and Calibrator and 4 Lots of the VITROS[®] Signal Reagent to assess the performance of the VITROS[®] HBeAg assay when tested with fresh VITROS[®] Signal Reagent and VITROS[®] Signal Reagent that has been stored at 2-8°C for 6 and 12 months. Reagent Packs, Calibrators and VITROS[®] HBeAg Controls were subjected to a period of simulated transport. This was to mimic the effects of the possible conditions that may occur during transport to customers. To simulate transport conditions, Reagent Packs, Calibrators and VITROS[®] HBe Controls were stored at 20°C for 2 days and then returned to 2-8°C prior to testing in the VITROS[®] Signal Reagent Study. Prior to the commencement of the 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.

VITROS[®] Signal Reagent has a shelf-life of 26 weeks. One (1) Master Lot of the VITROS[®] HBeAg Reagent Pack and Calibrator was tested with 4 lots of VITROS[®] Signal Reagent in the following ways: 2 lots of fresh VITROS[®] Signal Reagent were tested at the initial time point (month 0). These were then stored for 6 months at 2-8°C (aged), and tested again at the 6 month time point alongside a new, fresh lot of VITROS[®] Signal Reagent. They were then stored for another 6

months at 2-8°C, and were tested alongside another new, fresh lot of VITROS® Signal Reagent at month 12. Four runs were performed on each lot of VITROS® Signal Reagent at each time point. Each run contained duplicate determinations of the Calibrator and single determinations of the QC In-house Controls as those described in detail in section G.2.i.

All results met the predetermined acceptance criteria. No obvious and significant performance difference was observed between results generated using VITROS® Signal Reagent that is 6 and 12 months old (stored at 2-8°C) and fresh VITROS® Signal Reagent based on the review of the line data of the VITROS® Signal Reagent Stability Study.

The study data shows that the performance of the VITROS® HBeAg Reagent Pack and Calibrator is acceptable when tested with fresh VITROS® Signal Reagent and VITROS® Signal Reagent that is up to 12 months old when stored at 2-8°C.

H. Preservative Effectiveness Studies

The VITROS® HBeAg Assay Reagent (Reagent 0: G1222AR), HRP Conjugate Reagent (Reagent 1: G1222CJ), and Calibrator Reagent (G1220CLT) are formulated as instructed in the Manufacturing Batch Record and filtered through a microbial retentive filter to reduce the microbial levels. All formulations contain an anti-microbial agent that provides protection against adventitious contamination by microorganisms. The filtered bulk material is then dispensed into either plastic bottles or glass vials as instructed in the Dispensing Batch Record in a controlled environment.

Studies on the following three aspects of microbiological control were carried out:

- Determination of post-dispensing microbial load at expiration week 52 of the stability trial.
- Preservative concentration over a 52 week shelf-life.
- Preservative efficacy 52 weeks post formulation (The procedure is based on US Pharmacopoeia 23/NF 18; general chapter 51).

Evaluation of microbial load post-dispensing on three representative batches of each reagent has demonstrated that the microbial control procedures are appropriate to meet the acceptance criterion for a total aerobic count of 100 CFU /ml. Results are in the order of <10 CFU/ml.

It was confirmed that the concentration of 2- Chloracetimide, within three discrete batches of VITROS® HBeAg Assay Reagent, remained consistent over 52 weeks from the start of the stability trial. At expiration the 2- Chloracetamide concentration was at 0.46%, which is 4.5 times greater than the effective concentration of > or =0.1%. It was also confirmed that each VITROS® HBeAg HRP Conjugate and

Calibrator Reagent contains a higher preservative concentration than the MIC for the duration of a minimum of 52 weeks from the start of the stability trial.

The Preservative efficacy for VITROS[®] HBeAg Assay Reagent (G1222AR), HRP Conjugate Reagent (G1222CJ) and Calibrator Reagent (G1220CLT) assessed at Initial (week 0), 20 and 52 weeks post formulation have met the requirements of the Preservative Efficacy Testing Procedure, based on the US Pharmacopoeia 23/NF 18; general chapter 51. The preservative shelf lives supported are detailed in the table below and these dates are calculated from the start dates of reagent manufacture (i.e. date of dispense):

Status of Preservative Effectiveness Testing

Component	Lot tested at week 0, 20 and 52 time-points	Current supported Preservative shelf-life
G1222AR	1	52 weeks
G1222CJ	1	52 weeks
G1220CLT	32	52 weeks

I. Precision

VITROS[®] HBeAg Assay Reproducibility Study Number 1 (Precision) was conducted to evaluate the impact of the study day (day) and clinical site (site) on the precision estimate for the assay on the VITROS[®] ECi/ECiQ Immunodiagnostic System. The study utilized a 6 member panel with results ranging in decreasing order around the cutoff. Testing was performed at three clinical sites from October 21 through November 24, 2008.

The panel consisted of 6 members composed primarily of a defibrinated plasma (serum like) matrix and was tested at each site using a single reagent lot (Lot 8). The following table lists the ID number and the mean s/c value obtained for each panel member during the study.

Reproducibility 1 Precision Panel

Panel Member ID	Panel Member Designation	Mean S/C Value
R1HBEAG1	R1	2.20
R1HBEAG2	R2	2.01
R1HBEAG3	R3	1.65
R1HBEAG4	R4	1.07
R1HBEAG5	R5	0.85
R1HBEAG6	R6	0.57

Of the six panel members, R4 and R5 were the panel members with s/c ratios closest to the decision point (cutoff) of 1.00 s/c.

Appropriate calibration was performed and verified on Day 0 of the study. On each testing day, Quality Control testing was performed using the VITROS[®] HBeAg Controls (Lot 3) C1 (negative) and C2 (positive), and a daily unknown sample (DUHBEAG8, specific for reagent lot 8). The daily unknown sample was included to generate results that would be available in the event that assay troubleshooting was necessary.

Subsequent to acceptable QC results, 2 replicates of each panel member (R1 through R6) were run per day at each clinical site. Testing was performed for a total of 20 days over a 28-day period (including Day 28, the last in-calibration day of the 28-day calibration cycle). A total of 40 observations (two replicates for 20 days) were generated for each panel member at each of the 3 testing sites. A total of 720 observations (120 observations per panel member) obtained from the testing were included in the analysis.

Of the 6 panel members, R4 and R5 were the panel members with s/c ratios closest to the decision point (cutoff) of 1.00 s/c. For panel member R4, the mean s/c ratio was 1.07 with a range of 0.94 to 1.22. The between day, within day and total precision estimates for all sites were 6.2%. For panel member R5, the mean s/c ratio was 0.85 with a range of 0.71 to 1.01. The between day, within day and total precision estimates for all sites were 7.7%. The remaining panel members (R1, R2, R3 and R6) had mean s/c ratios further from the cutoff. For panel member R1, the mean s/c ratio was 2.20 with a range of 1.92 to 2.54. The between day, within day and total precision estimates for all sites were 7.1%. For panel member R2, the mean s/c ratio was 2.01 with a range of 1.69 to 2.32. The between day, within day and total precision estimates for all sites were 6.5%. For panel member R3, the mean s/c ratio was 1.65 with a range of 1.46 to 1.98. The between day, within day and total precision estimates for all sites were 8.4%. For panel member R6, the mean s/c ratio was 0.57 with a range of 0.45 to 0.69. The between day, within day and total precision estimates for all sites were 10.9%.

The results are shown below:

Site	Panel Member	No. of Obs.	No. of Days	Mean VITROS® HBeAg Test s/c Ratio	Repeatability*		Between Day**		Total***	
					SD	CV (%)	SD	CV (%)	SD	CV (%)
Site 1	R1	40	20	2.18	0.043	2.0	0.111	5.1	0.119	5.5
	R2	40	20	1.94	0.042	2.2	0.119	6.1	0.126	6.5
	R3	40	20	1.65	0.035	2.1	0.094	5.7	0.100	6.1
	R4	40	20	1.07	0.042	3.9	0.039	3.6	0.057	5.4
	R5	40	20	0.85	0.033	3.8	0.057	6.7	0.066	7.7
	R6	40	20	0.58	0.016	2.7	0.047	8.1	0.050	8.5
Site 2	R1	40	20	2.25	0.042	1.9	0.118	5.2	0.125	5.6
	R2	40	20	2.01	0.031	1.6	0.115	5.7	0.119	5.9
	R3	40	20	1.63	0.039	2.4	0.086	5.3	0.095	5.8
	R4	40	20	1.05	0.028	2.7	0.032	3.0	0.042	4.0
	R5	40	20	0.85	0.023	2.7	0.057	6.8	0.062	7.3
	R6	40	20	0.55	0.018	3.3	0.036	6.6	0.041	7.4
Site 3	R1	40	20	2.18	0.039	1.8	0.149	6.8	0.154	7.1
	R2	40	20	2.08	0.051	2.5	0.097	4.7	0.110	5.3
	R3	40	20	1.67	0.054	3.2	0.130	7.8	0.140	8.4
	R4	40	20	1.09	0.031	2.9	0.059	5.4	0.067	6.2
	R5	40	20	0.84	0.030	3.5	0.054	6.4	0.061	7.3
	R6	40	20	0.58	0.027	4.7	0.057	9.8	0.063	10.9

* Repeatability (within-day): Variability of the test performance from replicate-to-replicate

** Between-Day: Variability of the test performance from day-to-day

*** Total: Variability of the test performance combining the effects of within-day and between-day

J. Reproducibility

VITROS® HBeAg Assay Reproducibility Study Number 2 was carried out to evaluate the effect of multiple factors such as the clinical site, reagent lot and study day on the precision estimate for the assay on the VITROS® ECi/ECiQ Immunodiagnostic System. The study utilized a 6 member panel with results ranging in descending order around the cutoff. Testing was performed at three clinical sites from December 2 through December 18, 2008.

The Reproducibility 2 panel consisted of 6 members composed primarily of a defibrinated plasma (serum like) matrix and was tested at each site using 3 reagent lots (Lots 7, 8 and 9). The following table lists the ID numbers and the mean s/c value obtained for the 162 replicates of each panel member during the study:

Reproducibility 2 Panel

Panel Member ID	Panel Member Designation	Mean S/C Value
R2HBEAG1	R1	2.20
R2HBEAG2	R2	2.04
R2HBEAG3	R3	1.63
R2HBEAG4	R4	1.11
R2HBEAG5	R5	0.86
R2HBEAG6	R6	0.59

Of the 6 panel members, R4 and R5 were the panel members with s/c ratios closest to the decision point (cutoff) of 1.00 s/c.

Appropriate calibration was performed and verified for all 3 reagent lots on Day 0 of the study. On each testing day, Quality Control testing was performed using the VITROS[®] HBeAg Controls (Lot 3) C1 (negative) and C2 (positive) and a daily unknown sample (DUHBEAGX, where X corresponds to the reagent lot in use (7, 8 or 9)). The daily unknown sample was included to generate results that would be available in the event that assay troubleshooting was necessary.

Subsequent to acceptable QC results, 3 replicates of each panel member (R1, R2, R3, R4, R5 and R6) were run with each of 3 reagent lots per day, for a total of 6 days at each of 3 clinical sites. The testing was performed within one calibration cycle (between Days 1 and 14 of a single calibration, inclusive). The order of testing of the lots was rotated at each site over a designated 6-day period to ensure that the lots were equally distributed as the first, second, and third test in sequence, per the pre-determined testing schedule. The same schedule was used at each of the 3 testing sites. A total of 54 observations (3 replicates x 3 reagent lots x 6 days) were generated for each panel member at each of the 3 testing sites. A total of 972 observations (162 observations per panel member) obtained from the testing were included in the analysis.

Variance component analysis was performed to assess the precision estimates of individual components of variance to the total variability. The mean s/c ratio, SD, relative variance, precision estimate (CV (%)), and the number of observations (No. of Obs.) are presented in the following table:

Panel Member	Mean S/C	Source	Relative Variance (%)	SD	Precision Estimate CV (%)	No. of Obs.
R1	2.20	Between Site	0.0	0.000	0.0	162
		Between Lot	20.3	0.084	3.8	
		Between Day (Site)	31.6	0.105	4.8	
		Within Day	48.2	0.130	5.9	
		Total	100.0	0.187	8.5	
R2	2.04	Between Site	1.9	0.027	1.3	162
		Between Lot	26.5	0.101	4.9	
		Between Day (Site)	18.3	0.084	4.1	
		Within Day	53.3	0.143	7.0	
		Total	100.0	0.196	9.6	
R3	1.63	Between Site	3.6	0.023	1.4	162
		Between Lot	11.8	0.041	2.5	
		Between Day (Site)	15.7	0.047	2.9	
		Within Day	68.8	0.098	6.0	
		Total	100.0	0.118	7.3	
R4	1.11	Between Site	3.0	0.017	1.6	162
		Between Lot	36.0	0.060	5.4	
		Between Day (Site)	25.0	0.050	4.5	
		Within Day	36.0	0.060	5.4	
		Total	100.0	0.099	8.9	
R5	0.86	Between Site	5.9	0.019	2.2	162
		Between Lot	24.6	0.038	4.4	
		Between Day (Site)	37.8	0.047	5.5	
		Within Day	31.7	0.043	5.0	
		Total	100.0	0.076	8.9	
R6	0.59	Between Site	8.1	0.018	3.1	162
		Between Lot	25.4	0.033	5.5	
		Between Day (Site)	15.7	0.026	4.3	
		Within Day	50.8	0.046	7.8	
		Total	100.0	0.065	10.9	

K. Calibration Interval Study

VITROS® HBeAg Assay Calibration Interval Study was carried out to evaluate the performance of the assay on the VITROS® ECi/ECiQ Immunodiagnostic System within and beyond one calibration cycle. The study utilized a six member panel with results ranging in decreasing order around the cutoff. Testing was performed as part of Reproducibility Study 1 (Precision) at 3 clinical sites from October 21 through November 26, 2008. The panel was tested within 1 calibration interval (28 days). Additional testing was performed on Days 29 and 30 of the calibration cycle to show that the analyzer would still yield valid results beyond the end of a 28-day cycle.

The study utilized the VITROS® HBeAg assay Reproducibility 1 Precision Panel which consisted of 6 members composed primarily of a defibrinated plasma matrix (serum like). The panel was tested at each site using a single reagent lot (Lot 8). The following table lists the ID number and the mean s/c value obtained for the 132 replicates of each panel member during the study:

Calibration Interval Study

Panel Member ID	Panel Member Designation	Mean S/C Value
R1HBEAG1	R1	2.21
R1HBEAG2	R2	2.01
R1HBEAG3	R3	1.65
R1HBEAG4	R4	1.07
R1HBEAG5	R5	0.85
R1HBEAG6	R6	0.57

Of the 6 panel members, R4 and R5 were the panel members with s/c ratios closest to the decision point (cutoff) of 1.00 s/c.

Appropriate calibration was performed and verified on Day 0 of the study. On each testing day, Quality Control testing was performed using the VITROS® HBeAg Controls (Lot 3) C1 (negative) and C2 (positive), and a daily unknown sample (DUHBEAG8, specific for reagent lot 8). The daily unknown sample was included to generate results that would be available in the event that assay troubleshooting was necessary.

Subsequent to acceptable QC results, 2 replicates of each panel member (R1 through R6) were run per day at each clinical site. Testing was performed for a total of 20 days over a 28-day period (including Day 28, the last in-calibration day of the calibration cycle). Testing was also performed on Days 29 and 30 of the calibration cycle to show that the analyzer would still yield valid results beyond the end of a 28-day cycle.

The performance of the VITROS® HBeAg assay within and beyond one calibration interval was evaluated in this study. The least squares regression analyses were performed within site and across sites.

The study data demonstrated adequate performance of the VITROS® HBeAg assay throughout the calibration interval (28 days) and 2 days beyond the expiration of calibration. This data supports the calibration interval claim of 28 days for the VITROS® HBeAg assay.

L. Seroconversion Sensitivity Study

To evaluate the clinical sensitivity of the VITROS® HBeAg assay in the detection of HBeAg, commercially available hepatitis B seroconversion panels were tested, in parallel, with the VITROS® HBeAg assay and an FDA approved comparator HBeAg assay for the qualitative detection of HBeAg.

Seroconversion panel testing was performed as described in the VITROS® HBeAg assay Non-Clinical Testing Protocol 05-024-01-1222/1223/1203-P. On each testing day, a quality control run was performed consisting of VITROS® HBeAg Controls C1 (negative) and C2 (positive), and a daily unknown (DUHBEAG1 or DUHBEAG4). The daily unknown sample (a masked calibrator) was included to generate results that would be available in the event that assay troubleshooting was necessary. Panel members were tested subsequent to acceptable QC results.

Eleven (11) seroconversion panels were purchased from the vendors and provided to the testing site by Ortho Clinical Diagnostics, Inc. The panels were tested with the VITROS® HBeAg assay and the FDA approved comparator HBeAg assay. The panel members were stored and handled according to the requirements in the instructions for use for the VITROS® and FDA approved comparator assays.

The clinical sensitivity of each HBeAg assay was evaluated by determining the earliest detection of HBeAg in each panel. The decision point for the VITROS® assay was a signal to cutoff (s/c) ratio of 1.00. All panel members were initially tested in singleton with the VITROS® HBeAg assay. Each panel member was also tested in singleton with the FDA approved comparator assay, consistent with the manufacturer's instructions for use.

To evaluate the clinical sensitivity of the VITROS® HBeAg assay relative to the FDA approved comparator HBeAg assay, the test results from both assays were compared for each seroconversion panel. The bleed number, bleed date, the elapsed time in days from the first bleed, assay test results, and the interpretation of the results of the VITROS® and FDA approved comparator assays, are compared.

Results for the 11 panels are summarized in the table 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 both assays for each of the seroconversion panels.

HBeAg Seroconversion Panel Study - Summary Results

Days to Reactive HBeAg Result					
Panel ID	FDA Approved Comparator HBeAg Assay		VITROS [®] HBeAg Assay		Difference in Days to HBeAg Reactive Result
	⁻¹	⁺²	⁻¹	⁺²	Comparator minus VITROS [®]
11004	15	48	15	48	0
6278	12	16	12	16	0
6282	19	21	21	26	-5
6284	57	61	61	64	-3
6285	47	52	52	54	-2
6292	35	42	42	44	-2
PHM921	5	7	7	12	-5
PHM935	30	35	30	35	0
RP009	3	11	3	11	0
RP016	24	56	24	56	0
RP017	36	40	36	40	0

¹ Post bleed day of last negative result, usually denotes previous bleed from first positive/reactive result.

² Post bleed day of first positive/reactive result.

The VITROS[®] and FDA approved comparator HBeAg assays were in agreement with 6 of the 11 panels. The VITROS[®] HBeAg assay became reactive from 2 to 5 days (one bleed) later than the FDA approved comparator assay in the remaining 5 panels.

M. High Dose Hook Effect Study

A series of samples having HBeAg concentrations ranging from 0 to 7,668 PEI U/mL were assayed in the VITROS[®] HBeAg test. Singleton determinations of each sample were made with each of 2 Kit Lots and results used to construct a plot of result versus concentration. All samples having a concentration greater than that corresponding to the cut-off gave reactive results in the VITROS[®] HBeAg test, demonstrating that the high dose hook effect does not interfere with the qualitative determination of HBeAg. This was further verified by determination of 12 known reactive samples assayed neat and diluted. All of these samples and their dilutions gave clearly reactive results.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Multi-Center Prospective Clinical Studies in the United States (Population 1) and in India (Population 2)

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS[®] HBeAg test among individuals with signs or symptoms or biochemical manifestations (elevated liver function tests) of hepatitis and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, or known exposure events. Samples were obtained from 1976 subjects prospectively enrolled at four geographically separated collection sites within the United States (Population 1) located in Miami, FL (53.5%), Dallas, TX (14.3%), Newark, NJ (6.2%) and Chicago, IL (26.0%). Samples were also obtained from 311 subjects with signs or symptoms of hepatitis prospectively enrolled in an area in India with a high prevalence of viral hepatitis (Population 2).

The signs or symptoms documented for the subjects with signs or symptoms included fatigue, anorexia, malaise, nausea, jaundice, abdominal pain, dark urine, headache, vomiting, weight loss, hepatomegaly, Flu-like symptoms, depression, pruritis, arthralgia, fever, skin irritations, cirrhosis, myalgia, aching joints, diarrhea, itching, light colored stool, rashes, ecchymosis (bruise), enlarged spleen, ascites, and elevated liver function tests, such as alanine aminotransferase (ALT) or aspartate aminotransferase (AST) 1.5X upper limit of normal. The study population 1 also included asymptomatic individuals at high risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event, and individuals who have had serological testing for hepatitis ordered as an aspect of their medical care.

The HBV disease classification for each subject was determined by a single point serological assessment 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 laboratory study.

The subjects in Population 1 were 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 five to 89 years. Testing of these samples with the VITROS[®] HBeAg test occurred at diagnostic laboratories located in Miami, FL (34.4%), Port Jefferson, NY (25.3%) and St. Paul, MN, (40.4%). Agreement of the VITROS[®] HBeAg test was assessed relative to the FDA approved comparator HBeAg test and HBV disease classification using serum samples from the 1976 subjects in Population 1.

The subjects in Population 2 were Indian (100.0%). The group was 73.0% male and 27.0% female, and ranged in age from 18 to 90 years. Testing of these samples with the VITROS[®] HBeAg test occurred at diagnostic laboratories located in Miami, FL

(35.4%), St. Paul, MN, (33.8%) and Port Jefferson, NY (30.9%). Agreement of the VITROS[®] HBeAg test was assessed relative to the FDA approved comparator HBeAg test and HBV disease classification using serum samples from the 311 subjects in Population 2.

Approximately 65.6% (1296/1976) of the prospective subjects in Population 1 reported no recent or current signs or symptoms of hepatitis. Of these 1296 asymptomatic individuals, 45.8% were enrolled in Miami, FL, 19.1% were enrolled in Dallas, TX, 7.1% were enrolled in Newark, NJ, and 28.0% were enrolled in Chicago, IL. The group was Caucasian (20.0%), African American (49.5%), Hispanic (23.6%), and Asian (3.2%) with the remaining 3.7% represented by other ethnic groups. The group was 52.9% male and 47.1% female and ranged in age from 5 to 89 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS[®] HBeAg test was reactive in 0.8% (11/1296) of the individuals in this group. The percent VITROS[®] HBeAg reactive results observed in the asymptomatic population at each site was 1.0% at Miami, FL, 0.0% at Dallas, TX, 1.1% at Newark, NJ and 1.1% at Chicago, IL. The expected results for the VITROS[®] HBeAg test are presented in the table below:

Expected Results for the VITROS[®] HBeAg Test in Study Subjects without Signs or Symptoms of Hepatitis in Population 1 (N=1296)

Age Range	Gender	Reactive		Negative		Total ⁵
		N ¹	Percent ²	N ³	Percent ⁴	
≤ 15	Female	0	0.0	1	100	1
	Male	0	0.0	4	100	4
16-20	Female	0	0.0	24	100	24
	Male	0	0.0	11	100	11
21-30	Female	1	1.0	95	99.0	96
	Male	0	0.0	106	100	106
31-40	Female	1	0.7	134	99.3	135
	Male	4	2.4	162	97.6	166
41-50	Female	0	0.0	157	100	157
	Male	3	1.4	212	98.6	215
51-60	Female	1	0.8	121	99.2	122
	Male	1	0.8	126	99.2	127
61-70	Female	0	0.0	49	100	49
	Male	0	0.0	41	100	41
> 70	Female	0	0.0	26	100	26
	Male	0	0.0	14	100	14
Unknown	Female	0	0.0	1	100	1
	Male	0	0.0	1	100	1
Total		11	0.8	1285	99.2	1296

¹The total number (N) of subjects in each age range/gender category with reactive VITROS[®] HBeAg results.

²The total number (N) of subjects in each age range/gender category that are reactive; expressed as a percentage (%) of all subjects in that category.

³The total number (N) of subjects in each age range/gender category with negative VITROS[®] HBeAg results.

⁴The total number (N) of subjects in each age range/gender category that are negative; expressed as a percentage (%) of all subjects in that category.

⁵The total number (N) of subjects in each age range/gender category.

Approximately 34.4% (680/1976) of the prospective subjects in Population 1 reported recent or current signs or symptoms of hepatitis. Of these 680 symptomatic individuals, 68.4% were enrolled in Miami, FL, 5.0% were enrolled in Dallas, TX, 4.4% were enrolled in Newark, NJ, and 22.2% were enrolled in Chicago, IL. The group was Caucasian (16.8%), African American (53.8%), Hispanic (24.3%), and Asian (1.2%) with the remaining 3.9% represented by other ethnic groups. The group was 55.7% male and 44.3% female and ranged in age from 12 to 81 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS[®] HBeAg test was reactive in 3.5% (24/680) of the individuals in this group. The percent VITROS[®] HBeAg reactive results observed in the symptomatic population at each site was 4.3% at Miami, FL, 0.0% at Dallas, TX, 0.0% at Newark, NJ and 2.6% at Chicago, IL. The expected results for the VITROS[®]

HBeAg test in subjects from Population 1 with signs or symptoms of hepatitis are presented in the table below.

Expected Results for the VITROS[®] HBeAg Test in Study Subjects with Signs or Symptoms of Hepatitis in Population 1 (N= 680)

Age Range	Gender	Reactive		Negative		Total ⁵
		N ¹	Percent ²	N ³	Percent ⁴	
≤ 15	Female	0	0.0	1	100	1
16-20	Female	0	0.0	5	100	5
	Male	0	0.0	8	100	8
21-30	Female	1	2.7	36	97.3	37
	Male	1	3.0	32	97.0	33
31-40	Female	1	2.0	50	98.0	51
	Male	1	1.6	62	98.4	63
41-50	Female	3	3.2	90	96.8	93
	Male	8	6.0	126	94.0	134
51-60	Female	1	1.3	74	98.7	75
	Male	6	5.7	99	94.3	105
61-70	Female	0	0.0	27	100	27
	Male	2	6.5	29	93.5	31
> 70	Female	0	0.0	12	100	12
	Male	0	0.0	5	100	5
Total		24	3.5	656	96.5	680

¹The total number (N) of subjects in each age range/gender category with reactive VITROS[®] HBeAg results.

²The total number (N) of subjects in each age range/gender category that are reactive; expressed as a percentage (%) of all subjects in that category.

³The total number (N) of subjects in each age range/gender category with negative VITROS[®] HBeAg results.

⁴The total number (N) of subjects in each age range/gender category that are negative; expressed as a percentage (%) of all subjects in that category.

⁵The 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. The mean age of the population was 38.5 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. The VITROS[®] HBeAg test was reactive in 23.8% (74/311) of the individuals in this group. The expected results for the subjects in Population 2 are shown in the table below.

Expected Results for the VITROS[®] HBeAg Test in Study Subjects with Signs or Symptoms of Hepatitis in Population 2 (N=311)

Age Range	Gender	Reactive		Negative		Total ⁵
		N ¹	Percent ²	N ³	Percent ⁴	
18-20	Female	2	28.6	5	71.4	7
	Male	3	15.0	17	85.0	20
21-30	Female	9	26.5	25	73.5	34
	Male	8	16.3	41	83.7	49
31-40	Female	12	38.7	19	61.3	31
	Male	14	22.2	49	77.8	63
41-50	Female	3	37.5	5	62.5	8
	Male	11	19.0	47	81.0	58
51-60	Female	1	33.3	2	66.7	3
	Male	7	24.1	22	75.9	29
61-70	Female	0	0.0	1	100	1
	Male	4	57.1	3	42.9	7
> 70	Male	0	0.0	1	100	1
Total		74	23.8	237	76.2	311

¹The total number (N) of subjects in each age range/gender category with reactive VITROS[®] HBeAg results.

²The total number (N) of subjects in each age range/gender category that are reactive; Expressed as a percentage (%) of all subjects in that category.

³The total number (N) of subjects in each age range/gender category with negative VITROS[®] HBeAg results.

⁴The total number (N) of subjects in each age range/gender category that are negative; Expressed as a percentage (%) of all subjects in that category.

⁵The total number (N) of subjects in each age range/gender category.

1. Sample Classification

The clinical study data were analyzed following the assignment of HBV disease classifications based upon the positive (+) / negative (-) patterns for the 6 HBV serological markers. The tables below summarize how these classifications were derived. There were 35 unique marker profiles observed among the subjects in Population 1 and 18 unique marker profiles observed among the subjects in Population 2 during the VITROS[®] HBeAg clinical study.

HBV Marker Profiles and HBV Disease Classification in Population 1

FDA Approved HBsAg ^{1,2,3}	FDA Approved HBeAg	FDA Approved aHBc IgM	FDA Approved aHBc Total	FDA Approved aHBe ³	FDA Approved aHBs ³ ≥10 mIU/mL	HBV Disease Classification
+	+	+	+	-	-	Acute
+	+	-	-	-	-	Acute
+	-	+	+	+	-	Acute
+	-	-	-	-	-	Acute
+	+	-	+	-	-	Chronic
+	+	-	+	-	I	Chronic
+	-	-	+	+	+	Chronic
+	-	-	+	+	-	Chronic
+	-	-	+	+	I	Chronic
+	-	-	+	-	+	Chronic
+	-	-	+	-	-	Chronic
+	-	-	+	I	-	Chronic
-	-	+	+	+	+	Early Recovery
-	-	+	+	+	-	Early Recovery
-	-	-	+	+	-	Early Recovery
-	-	-	+	I	-	Early Recovery
-	-	-	+	+	+	Recovery
-	-	-	+	I	+	Recovery
-	-	-	-	+	+	Recovery
-	-	-	+	-	+	Recovered
-	-	-	+	-	-	Recovered
-	-	-	+	-	I	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	I	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected with HBV
+	+	-	-	-	+	Uninterpretable
+	-	-	-	-	+	Uninterpretable
+	-	-	-	-	I	Uninterpretable
-	+	-	+	-	+	Uninterpretable
-	+	-	+	I	+	Uninterpretable
-	+	-	-	-	-	Uninterpretable
-	-	-	+	+	I	Uninterpretable
-	-	-	+	I	I	Uninterpretable
-	-	-	-	+	-	Uninterpretable
I	-	-	+	-	-	Uninterpretable

¹ Positive = FDA approved HBsAg test positive or reactive and confirmed by neutralization.

² Negative = FDA approved HBsAg test negative or not confirmed by neutralization.

³ I = Indeterminate result.

HBV Marker Profiles and HBV Disease Classification in Population 2

FDA Approved HBsAg ^{1,2}	FDA Approved HBeAg	FDA Approved aHBc IgM	FDA Approved aHBc Total	FDA Approved aHBe	FDA Approved aHBs ≥10 mIU/mL	HBV Disease Classification
+	+	+	+	+	-	Acute
+	+	+	+	-	-	Acute
+	-	+	+	+	+	Acute
+	-	+	+	+	-	Acute
+	-	+	+	-	-	Acute
+	-	-	-	-	-	Acute
+	+	-	+	+	-	Chronic
+	+	-	+	-	+	Chronic
+	+	-	+	-	-	Chronic
+	-	-	+	+	+	Chronic
+	-	-	+	+	-	Chronic
+	-	-	+	-	-	Chronic
-	-	+	+	+	-	Early Recovery
-	-	-	+	-	+	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected with HBV
+	+	-	-	+	+	Uninterpretable
+	-	-	-	-	+	Uninterpretable

¹ Positive = FDA approved HBsAg test positive or reactive and confirmed by neutralization.

² Negative = FDA approved HBsAg test negative or not confirmed by neutralization.

2. Comparison of Results

The table below compares the VITROS[®] HBeAg results with the FDA approved comparator HBeAg results by HBV disease classification for the subjects in Population 1.

Comparison of VITROS[®] HBeAg Test Results with FDA Approved Comparator HBeAg Test Results by HBV Classification in Population 1 (N=1976)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Acute	12	0	0	6	0	0	18
Chronic	16	2 ²	0	52	0	0	70
Early Recovery	0	0	0	57	0	0	57
Recovery	0	0	1 ³	213	0	0	214
Recovered	0	0	3 ⁴	216	0	0	219
HBV Vaccine Response	0	0	1 ³	313	0	0	314
Not Previously Infected with HBV	0	0	1 ³	1044	0	0	1045
Uninterpretable	1	13 ^{1,2}	0	25	0	0	39
Overall	29	15	6	1926	0	0	1976

¹ These 13 samples were HBsAg negative and 10 of the 13 had the FDA approved comparator HBeAg test as the only positive HBV marker. This suggests that the FDA approved comparator HBeAg test results are falsely positive and that the VITROS[®] HBeAg negative results are likely correct.

² These 15 FDA approved comparator HBeAg positive samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

³ These 3 VITROS[®] HBeAg reactive samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

⁴ Two (2) of these 3 VITROS[®] HBeAg reactive samples were confirmed with an in-house HBeAg confirmatory neutralization test.

The table below compares the VITROS[®] HBeAg results with the FDA approved comparator HBeAg results by HBV disease classification for the subjects in Population 2.

Comparison of VITROS[®] HBeAg Test Results with FDA Approved Comparator HBeAg Test Results by HBV Disease Classification in Population 2 (N=311)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Acute	7	1 ¹	2 ³	90	0	0	100
Chronic	41	13 ²	20 ⁴	111	0	0	185
Early Recovery	0	0	0	1	0	0	1
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	3 ³	14	0	0	17
Uninterpretable	1	0	0	1	0	0	2
Overall	49	14	25	223	0	0	311

¹ This FDA approved comparator HBeAg positive sample was confirmed with an in-house HBeAg confirmatory neutralization test.

² Eight (8) of these 13 FDA approved comparator HBeAg positive samples (61.5%) were confirmed and five (38.5%) were not confirmed with an in-house HBeAg confirmatory neutralization test.

³ These 5 VITROS[®] HBeAg reactive samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

⁴ Fifteen (15) of these 20 VITROS[®] HBeAg reactive samples had sufficient volume for testing with an in-house HBeAg confirmatory neutralization test. One (1) was confirmed and 14 (93.3%) were not confirmed.

3. Percent Agreement

Positive and negative percent agreement between the VITROS[®] HBeAg test and the FDA approved comparator HBeAg test were calculated for subjects in Population 1 (N=1976) with the various HBV disease classifications. The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals.

Positive and Negative Percent Agreement between the VITROS[®] HBeAg and FDA Approved Comparator HBeAg Tests by HBV Classification in Population 1 (N=1976)

HBV Classification	Positive Percent Agreement % (N/Total)	95% Exact Confidence Interval	Negative Percent Agreement % (N/Total)	95% Exact Confidence Interval
Acute	100.0% (12/12)	73.54% - 100.0%	100.0% (6/6)	N/A ¹
Chronic	88.89% (16/18) ²	65.29% - 98.62%	100.0% (52/52)	93.15% - 100.0%
Early Recovery	N/A (0/0)	N/A	100.0% (57/57)	93.73% - 100.0%
Recovery	N/A (0/0)	N/A	99.53% (213/214) ³	97.42% - 99.99%
Recovered	N/A (0/0)	N/A	98.63% (216/219) ⁴	96.05% - 99.72%
HBV Vaccine Response	N/A (0/0)	N/A	99.68% (313/314) ³	98.24% - 99.99%
Not Previously Infected with HBV	N/A (0/0)	N/A	99.90% (1044/1045) ³	99.47% - 100.0%
Uninterpretable	7.14% (1/14) ²	0.18% - 33.87%	100.0% (25/25)	86.28% - 100.0%

¹ Confidence intervals calculated on small numbers are not meaningful.

² The 15 FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

³ The 3-VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

⁴ Two (2) of the 3 VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) samples were confirmed with an in-house HBeAg confirmatory neutralization test.

Positive percent agreement with the FDA approved comparator HBeAg test was determined by dividing the number of reactive VITROS[®] HBeAg results by the number of subjects positive with the FDA approved comparator HBeAg test. As a result of this study, the positive percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in samples with an acute HBV disease classification was 100% (12/12 with a 95% exact confidence interval [CI] of 73.54% to 100.0%). The positive percent agreement in samples with a chronic HBV disease classification was 88.89% (16/18; CI = 65.29% to 98.62%).

Negative percent agreement with the FDA approved comparator HBeAg test was determined by dividing the number of negative VITROS[®] HBeAg results by the number of subjects negative with the FDA approved comparator HBeAg test. As a result of this study, the negative percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in samples with an acute HBV disease classification was 100% (6/6; confidence intervals calculated on small numbers are not meaningful). The negative percent agreement in samples with a chronic HBV disease classification was 100.0% (52/52; CI = 93.15% to 100.0%).

Positive and negative percent agreement between the VITROS[®] HBeAg test and the FDA approved comparator HBeAg test were also calculated for subjects in Population 2 (N=311) with the various HBV disease classifications. The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals.

Positive and Negative Percent Agreement between the VITROS[®] HBeAg and FDA Approved Comparator HBeAg Tests by HBV Disease Classification in Population 2 (N=311)

HBV Disease Classification	Positive Percent Agreement %(N/Total)	95% Exact Confidence Interval	Negative Percent Agreement %(N/Total)	95% Exact Confidence Interval
Acute	87.50% (7/8) ²	N/A ¹	97.83% (90/92) ⁴	92.37% - 99.74%
Chronic	75.93% (41/54) ³	62.36% - 86.51%	84.73% (111/131) ⁵	77.41% - 90.42%
Early Recovery	N/A (0/0)	N/A	100.0% (1/1)	N/A ¹
Recovered	N/A (0/0)	N/A	100.0% (3/3)	N/A ¹
HBV Vaccine Response	N/A (0/0)	N/A	100.0% (3/3)	N/A ¹
Not Previously Infected with HBV	N/A (0/0)	N/A	82.35% (14/17) ⁴	56.57% - 96.20%
Uninterpretable	100.0% (1/1)	N/A ¹	100.0% (1/1)	N/A ¹

¹ Confidence intervals calculated on small numbers are not statistically meaningful.

² This FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) sample was confirmed with an in-house HBeAg confirmatory neutralization test.

³ Eight (8) of the 13 FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) samples (61.5%) were confirmed and five (38.5%) were not confirmed with an in-house HBeAg confirmatory neutralization test.

⁴ The 5 VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

⁵ Fifteen (15) of the 20 VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) samples had sufficient volume for testing with an in-house confirmatory neutralization test. One (1) was confirmed and 14 (93.3%) were not confirmed.

Positive percent agreement with the FDA approved comparator HBeAg test was determined by dividing the number of reactive VITROS[®] HBeAg results by the number of subjects positive with the FDA approved comparator HBeAg test. As a result of this study, the positive percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in samples with an acute HBV disease classification was 87.50% (7/8; confidence intervals calculated on small numbers are not meaningful). The positive percent agreement in samples with a chronic HBV disease classification was 75.93% (41/54; CI = 62.36% to 86.51%).

Negative percent agreement with the FDA approved comparator HBeAg test was determined by dividing the number of negative VITROS[®] HBeAg results by the

number of subjects negative with the FDA approved comparator HBeAg test. As a result of this study, the negative percent agreement of the VITROS® HBeAg test with the FDA approved comparator HBeAg test in samples with an acute HBV disease classification was 97.83% (90/92; CI = 92.37% to 99.74%). The negative percent agreement in samples with a chronic HBV disease classification was 84.73% (111/131; CI = 77.41% to 90.42%).

4. Potentially Cross-Reacting Subgroups

Samples with evidence of hepatitis A virus infection (HAV) or hepatitis C virus infection (HCV) were identified in a population of 1976 samples prospectively collected from subjects in the U.S with signs or symptoms of, or at risk for, viral hepatitis (Population 1). The tables below compare VITROS® HBeAg results with FDA approved comparator HBeAg results according to the HBV disease classifications assigned to the study subjects.

Comparison of VITROS® and FDA Approved Comparator HBeAg Test Results and HBV Disease Classification among Anti-HAV IgM Reactive Study Subjects in Population 1 (N=5)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS® HBeAg Test Result		VITROS® HBeAg Test Result		VITROS® HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Recovered	0	0	0	1	0	0	1
Not Previously Infected with HBV	0	0	0	4	0	0	4
Overall	0	0	0	5	0	0	5

Comparison of VITROS® and FDA Approved Comparator HBeAg Test Results and HBV Disease Classification among Anti-HCV Reactive Study Subjects in Population 1 (N=398)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS® HBeAg Test Result		VITROS® HBeAg Test Result		VITROS® HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Acute	2	0	0	1	0	0	3
Chronic	1	0	0	8	0	0	9
Early Recovery	0	0	0	34	0	0	34
Recovery	0	0	0	61	0	0	61
Recovered	0	0	0	98	0	0	98
HBV Vaccine Response	0	0	0	46	0	0	46
Not Previously Infected with HBV	0	0	0	135	0	0	135
Uninterpretable	0	2 ¹	0	10	0	0	12
Overall	3	2	0	393	0	0	398

¹ These 2 FDA approved comparator HBeAg positive samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

Samples with evidence of hepatitis A virus infection (HAV) or hepatitis C virus infection (HCV) were identified in a population of 311 samples prospectively collected from subjects in an area in India with a high prevalence of viral hepatitis (Population 2). The tables below compare VITROS[®] HBeAg results with FDA approved comparator HBeAg results according to the HBV disease classifications assigned to the study subjects.

Comparison of VITROS[®] and FDA Approved Comparator HBeAg Test Results and HBV Disease Classification among Anti-HAV IgM Reactive Subjects in Population 2 (N=28)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Acute	0	0	1 ¹	16	0	0	17
Chronic	0	0	0	1	0	0	1
HBV Vaccine Response	0	0	0	3	0	0	3
Not Previously Infected with HBV	0	0	0	6	0	0	6
Uninterpretable	1	0	0	0	0	0	1
Overall	1	0	1	26	0	0	28

¹ This VITROS[®] HBeAg reactive sample did not confirm with an in-house HBeAg confirmatory neutralization test.

Comparison of VITROS[®] and FDA Approved Comparator HBeAg Test Results and HBV Disease Classification among Anti-HCV Reactive Subjects in Population 2 (N=90)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		VITROS [®] HBeAg Test Result		
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Acute	0	1 ¹	0	57	0	0	58
Chronic	8	11 ²	0	13	0	0	32
Overall	8	12	0	70	0	0	90

¹ This FDA approved comparator HBeAg positive sample was confirmed with an in-house HBeAg confirmatory neutralization test.

² These 11 FDA approved comparator HBeAg positive samples were tested with an in-house HBeAg confirmatory neutralization test. Eight (8) were confirmed and 3 were not confirmed.

5. Clinically Diagnosed Chronic HBV Infection in Population 1

The performance of the VITROS[®] HBeAg assay was evaluated among 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 was detected in the current sample, and the individual's medical record had documentation of the presence of HBsAg or HBV DNA or HBeAg (by FDA approved method) at least 6 months prior to the current positive HBsAg sample
- HBsAg was detected in the current sample, and the individual's medical record had documentation of diagnosis of HBV infection at least 6 months prior to the current positive HBsAg sample
- The individual's medical record documented the presence of HBsAg or HBV DNA or HBeAg (by FDA approved method) 2 times at least 6 months apart

Based on the above definitions, 36 individuals from Population 1 were documented to have a chronic HBV infection. Of the 36 samples, 13 (36.1%) were positive with the FDA approved comparator HBeAg assay and 11 (30.6%) were reactive with the VITROS[®] HBeAg test. The table below summarizes the positive and negative percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in samples from these 36 subjects with clinically documented chronic HBV infection.

Performance of the VITROS[®] HBeAg Test in Samples from Subjects with Clinically Documented Chronic HBV Infection in Population 1 (N=36)

Population	Positive Percent Agreement % (N/Total)	95% Exact Confidence Intervals	Negative Percent Agreement % (N/Total)	95% Exact Confidence Intervals
Subjects with Clinically Documented Chronic HBV Infection	84.62% (11/13) ¹	54.55% - 98.08%	100.0% (23/23)	85.18% - 100.0%

¹ The 2 FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

B. Additional Clinical Study Testing Samples Prospectively Collected from Subjects with Clinically Documented Chronic HBV Infection in Russia

An additional study was conducted to further evaluate the clinical performance of the VITROS[®] HBeAg test among individuals with clinically documented chronic HBV infection. A total of 40 samples were obtained from a vendor. The vendor prospectively collected chronic HBV samples from a variety of hospitals and clinics in Moscow, Russia. All samples were collected with proper informed consent under an Ethical Committee approved protocol. Samples were subsequently shipped to Los Angeles, CA, USA (via OCD authorized sample shipment courier with a replenishment of dry ice capability.). Samples were unlinked/de-identified to OCD. All samples were relabeled with barcodes provided by the vendor as per OCD specifications. All samples have 2 HBsAg (and/or HBV DNA) positive test results at least 6 months apart (The vendor provided to OCD a signed copy of a Certificate of Analysis (CoA) containing information regarding each subject's age, gender,

ethnicity, date of blood draw, and at a minimum, supporting documentation for 2 positive HBsAg results within a six-month time frame).

Demographic information for these subjects is presented in the table below.

Testing Site	CFLD N(%) ¹
TOTAL	40 (100.0)
Male	34 (85.0)
Female	6 (15.0)
11 - 20	0 (0)
21 - 30	20 (50.0)
31 - 40	10 (25.0)
41 - 50	4 (10.0)
> 50	6 (15.0)
Caucasian	40 (100.0)

¹ The total number (N) of subjects in each category; Expressed as a percentage (%) of analyzed subjects (N=40)

Of the 40 samples, 10 (25.0%) were positive with the FDA approved comparator HBeAg assay and 10 (25.0%) were reactive with the VITROS[®] HBeAg test. The table below summarizes the positive and negative percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in samples from these 40 subjects with clinically documented chronic HBV infection.

Performance of the VITROS[®] HBeAg Test in Prospective Samples from Subjects with Clinically Documented Chronic HBV Infection in Russia (N=40)

Population	Positive Percent Agreement % (N/Total)	95% Exact Confidence Intervals	Negative Percent Agreement % (N/Total)	95% Exact Confidence Intervals
Subjects with Clinically Documented Chronic HBV Infection	80.00% (8/10) ¹	44.39% - 97.48%	93.33% (28/30) ²	77.93% - 99.18%

¹ The 2 FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) samples were confirmed with an in-house HBeAg confirmatory neutralization test.

² The 2 VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) sample were not confirmed with an in-house HBeAg confirmatory neutralization test.

C. Clinical Study Testing Archived Samples Collected from Subjects with Serologically Determined Chronic HBV Infection

Archived samples were obtained from a vendor and used to further assess the performance of the VITROS[®] HBeAg test in subjects with chronic HBV infection. The HBV disease classification for these archived samples was determined by a

single point serological assessment using a hepatitis marker profile consisting of FDA approved tests for the detection of HBsAg, HBeAg, anti-HBc Total, anti-HBc IgM, anti-HBe and anti-HBs (quantitative). A total of 277 samples with a marker profile suggestive of chronic HBV infection were tested with the VITROS[®] HBeAg test. The FDA approved comparator HBeAg test was positive with 76 of the 277 samples (27.4%). The VITROS[®] HBeAg test was reactive with 77 of the 277 samples (27.8%). All FDA approved comparator HBeAg test or VITROS[®] HBeAg test positive samples were confirmed with an in-house HBeAg confirmatory neutralization test. The table below summarizes the positive and negative percent agreement of the VITROS[®] HBeAg test with the FDA approved comparator HBeAg test in subjects with serologically determined chronic HBV infection.

Performance of the VITROS[®] HBeAg Test in Archived Samples with Serologically Determined Chronic HBV Infection (N=277)

Population	Positive Percent Agreement % (N/Total)	95% Exact Confidence Intervals	Negative Percent Agreement % (N/Total)	95% Exact Confidence Intervals
Subjects with Serologically Determined Chronic HBV Infection	98.68% (75/76) ¹	92.89% - 99.97%	99.00% (199/201) ²	96.45% - 99.88%

¹ The FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) sample was confirmed with the in-house HBeAg confirmatory neutralization test.

² The 2 VITROS[®] HBeAg reactive (FDA approved comparator HBeAg negative) samples were confirmed with the in-house HBeAg confirmatory neutralization test.

The positive percent agreement between the VITROS[®] and FDA approved comparator HBeAg tests among samples with serological marker profiles of chronic HBV infection was 98.68% (75/76; CI = 92.89% to 99.97%). The negative percent agreement between the VITROS and FDA approved comparator HBeAg tests was 99.00% (199/201; CI = 96.45% to 99.88%).

D. Multi-Center Prospective Clinical Study in 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 VITROS[®] HBeAg test. Of the 245 prospectively collected samples, 75.1% were obtained in Florida and 24.9% were obtained in Texas. In this population, 18.8% of the pregnant women were in their first trimester, 40.8% were in the second trimester, and 40.4% were in the third trimester of pregnancy. The table below provides a breakdown of the study population.

Demographic Profiles of Pregnant Women at High Risk for Viral Hepatitis (N=245)

Collection Site	Florida N(%) ¹	Texas N(%) ¹	Total N(%) ²
TOTAL	184 (75.1)	61 (24.9)	245 (100.0)
AGE (Years)			
11-20	30 (16.3)	22 (36.1)	52 (21.2)
21-30	76 (41.3)	29 (47.5)	105 (42.9)
31-40	70 (38.0)	9 (14.8)	79 (32.2)
41-50	8 (4.3)	1 (1.6)	9 (3.7)
> 50	0 (0.0)	0 (0.0)	0 (0.0)
ETHNICITY			
Caucasian	4 (2.2)	10 (16.4)	14 (5.7)
African-American	48 (26.1)	23 (37.7)	71 (29.0)
Hispanic	108 (58.7)	24 (39.3)	132 (53.9)
Asian	1 (0.5)	0 (0.0)	1 (0.4)
Indian	1 (0.5)	3 (4.9)	4 (1.6)
Haitian	16 (8.7)	0 (0.0)	16 (6.5)
Other	4 (2.2)	0 (0.0)	4 (1.6)
Unknown	2 (1.1)	1 (1.6)	3 (1.2)
TRIMESTER			
First	7 (3.8)	39 (63.9)	46 (18.8)
Second	86 (46.7)	14 (23.0)	100 (40.8)
Third	91 (49.5)	8 (13.1)	99 (40.4)

¹ The number (N) of subjects at each site; Expressed as a percentage (%) of analyzed subjects at each site.

² The total number (N) of subjects in each category; Expressed as a percentage (%) of enrolled subjects (N=245).

The data were analyzed following the assignment of HBV disease classifications based upon the positive (+) / negative (-) patterns for the 6 HBV serological markers. The table below summarizes how these classifications were derived. There were 12 unique marker profiles observed among the pregnant women at high risk for hepatitis. Due to insufficient volume, the 6 marker profile for one sample from a 21 year old Hispanic woman in the first trimester could not be completed, leaving 244 samples for analysis.

HBV Marker Profiles and HBV Disease Classifications among Pregnant Women at High Risk for Hepatitis

FDA Approved HBsAg ^{1,2}	FDA Approved HBeAg	FDA Approved aHBc IgM	FDA Approved aHBc Total	FDA Approved aHBe	FDA Approved aHBs ³ ≥10 mIU/ML	HBV DISEASE CLASSIFICATION
+	+	-	+	-	-	Chronic
+	-	-	+	+	-	Chronic
-	-	+	+	+	+	Early Recovery
-	-	-	+	+	-	Early Recovery
-	-	-	+	+	+	Recovery
-	-	-	+	-	+	Recovered
-	-	-	+	-	-	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	I	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected with HBV
-	+	-	-	-	+	Uninterpretable
-	+	-	-	-	-	Uninterpretable

¹ Positive = FDA Approved HBsAg test positive or reactive and confirmed by neutralization.

² Negative = FDA Approved HBsAg test negative or not confirmed by neutralization.

³ I = Indeterminate result.

The table below compares the VITROS[®] and FDA approved comparator HBeAg test results among the population of pregnant women by trimester. None of the samples had indeterminate results with the FDA approved comparator HBeAg test.

VITROS[®] and FDA Approved Comparator HBeAg Test Results among Pregnant Women at High Risk for Hepatitis (N=244): Results by Trimester

VITROS [®] HBeAg Test Result	First Trimester			Second Trimester			Third Trimester		
	FDA Approved Comparator HBeAg Test Result		Total	FDA Approved Comparator HBeAg Test Result		Total	FDA Approved Comparator HBeAg Test Result		Total
	+	-		+	-		+	-	
Reactive	1	0	1	0	0	0	0	0	0
Negative	0	44	44	0	100	100	3 ¹	96	99
Total	1	44	45	0	100	100	3	96	99

¹ These 3 FDA approved comparator HBeAg positive samples were tested with an in-house HBeAg confirmatory neutralization test. One (1) was confirmed and two were not confirmed.

The table below compares the VITROS[®] and FDA approved comparator HBeAg test results for the population of pregnant women at high risk for hepatitis.

VITROS[®] vs. FDA Approved Comparator HBeAg Test Results in Pregnant Women at High Risk for Hepatitis (N=244)

VITROS [®] HBeAg Test Result	FDA Approved Comparator HBeAg Test Result			Total N
	Positive N	Negative N	Indeterminate N	
Reactive	1	0	0	1
Negative	3 ¹	240	0	243
TOTAL	4	240	0	244

¹ These 3 FDA approved comparator HBeAg positive samples were tested with an in-house HBeAg confirmatory neutralization test. One (1) was confirmed and 2 were not confirmed.

The table below compares the VITROS[®] HBeAg test results with the FDA approved comparator HBeAg test results by HBV Disease Classification.

Comparison of VITROS[®] HBeAg Test Results with FDA Approved Comparator HBeAg Test Results by HBV Disease Classification among Pregnant Women at High Risk for Hepatitis (N=244)

HBV Disease Classification	FDA Approved Comparator HBeAg Test Result						Total
	Positive		Negative		Indeterminate		
	VITROS [®] HBeAg Test Result	VITROS [®] HBeAg Test Result	VITROS [®] HBeAg Test Result	VITROS [®] HBeAg Test Result	VITROS [®] HBeAg Test Result	VITROS [®] HBeAg Test Result	
	Reactive	Negative	Reactive	Negative	Reactive	Negative	
Chronic	1	1 ³	0	1	0	0	3
Early Recovery	0	0	0	3	0	0	3
Recovery	0	0	0	10	0	0	10
Recovered	0	0	0	11	0	0	11
HBV Vaccine Response	0	0	0	27	0	0	27
Not Previously Infected with HBV	0	0	0	188	0	0	188
Uninterpretable	0	2 ^{1,2}	0	0	0	0	2
Overall	1	3	0	240	0	0	244

¹ These 2 VITROS[®] negative discordant samples were retested a single time with the VITROS[®] and FDA approved comparator HBeAg tests. Both samples remained negative with the VITROS[®] test. The FDA approved comparator test s/c results for the first sample changed from 3.318 to 0.183, and the second from 1.370 to 0.183. The two samples were also HBsAg negative.

² These 2 FDA approved comparator HBeAg positive samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

³ This FDA approved comparator HBeAg positive sample was confirmed with an in-house HBeAg confirmatory neutralization test.

Positive and negative percent agreement between the VITROS[®] HBeAg test and the FDA approved comparator HBeAg test were calculated for the pregnant women at high risk for hepatitis (N=244) with the various HBV disease classifications. The

table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals, where appropriate.

Positive and Negative Percent Agreement between the VITROS[®] HBeAg and FDA Approved Comparator HBeAg Tests by HBV Disease Classification among Pregnant Women at High Risk for Hepatitis (N=244)

HBV Disease Classification	Positive Percent Agreement %(N/Total)	95% Exact Confidence Interval	Negative Percent Agreement %(N/Total)	95% Exact Confidence Interval
Chronic	50.00% (1/2) ²	N/A ¹	100.0% (1/1)	N/A ¹
Early Recovery	N/A (0/0)	N/A	100.0% (3/3)	N/A ¹
Recovery	N/A (0/0)	N/A	100.0% (10/10)	69.15% - 100.0%
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%
Uninterpretable	0.00% (0/2) ³	N/A ¹	N/A (0/0)	N/A

¹ Confidence intervals calculated on small numbers are not meaningful.

² The FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) sample was confirmed with an in-house HBeAg confirmatory neutralization test.

³ The 2 FDA approved comparator HBeAg positive (VITROS[®] HBeAg negative) samples were not confirmed with an in-house HBeAg confirmatory neutralization test.

There were no acute samples among the samples collected from pregnant women at high risk for hepatitis. The positive percent agreement between the VITROS[®] and FDA approved comparator HBeAg tests in the samples with a chronic HBV disease classification was 50% (1/2). The negative percent agreement was 100% (1/1) with the one FDA approved comparator HBeAg negative chronic sample. The VITROS[®] and FDA approved comparator HBeAg tests were in agreement in 100.0% of the FDA approved comparator test negative samples.

E. Prospective Clinical Study in Pediatric Subjects

Performance of the VITROS[®] HBeAg test was also determined using prospective samples from a population of pediatric subjects in Florida at high risk for viral hepatitis (N=165). The group was 47.9% male and 52.1% female, and the subjects' ages ranged from 2 through 21 years. The samples were tested with the VITROS[®] HBeAg test. The table below provides a breakdown of the study population.

Demographic Profiles of Pediatric Study Subjects (N=165)

	Total N(%) ¹
TOTAL	165 (100.0)
GENDER	
Male	79 (47.9)
Female	86 (52.1)
AGE (Years)	
2-4	24 (14.5)
5-8	35 (21.2)
9-12	37 (22.4)
13-16	35 (21.2)
17-21	34 (20.6)
ETHNICITY	
Hispanic or Latino	70 (42.4)
Non-Hispanic or Latino	95 (57.6)
RACE	
White	77 (46.7)
Black or African American	49 (29.7)
Other	39 (23.6)
RISK FACTOR	
No Risk Factor(s) ²	36 (21.8)
Risk Factor(s)	129 (78.2)
SIGNS AND SYMPTOMS	
No Signs and Symptoms	103 (62.4)
Signs and Symptoms	62 (37.6)

¹ The total number (N) of subjects in each category; Expressed as a percentage (%) of analyzed subjects (N = 165).

² Subjects with no acknowledged risk factor(s) were enrolled at the discretion of the principal investigator or designee based on individual's inclusion in or association with other individuals or groups at risk for viral hepatitis in that geographic area

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The table below compares the VITROS[®] HBeAg results with the FDA approved comparator HBeAg results for the pediatric subjects.

VITROS[®] vs. FDA Approved Comparator HBeAg Test Results in Pediatric Subjects (N=165)

VITROS [®] HBeAg Test Result	FDA Approved Comparator HBeAg Test Result			Total N
	Positive N	Negative N	Indeterminate N	
Reactive	0	0	0	0
Negative	0	165	0	165
TOTAL	0	165	0	165

The table below summarizes the percent agreement between the VITROS[®] HBeAg test and the FDA approved comparator HBeAg test for the pediatric population. The table provides the 95% exact confidence intervals.

Positive and Negative Agreement of the VITROS[®] HBeAg Test with the FDA Approved Comparator HBeAg Test in Pediatric Subjects - 95% Exact Confidence Intervals

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% (165/165) concordance (95% exact confidence interval 97.79% - 100.0%) between the VITROS[®] and FDA approved comparator HBeAg tests. None of the 165 samples were reactive with either the VITROS[®] HBeAg assay or the FDA approved comparator HBeAg test. There were no indeterminate FDA approved comparator test results for the pediatric population.

F. Testing of the VITROS[®] HBeAg Test with HBeAg Spiked Pediatric Samples

All of the 165 prospectively collected samples from high risk pediatric subjects between the ages of 2 and 21 years tested in the VITROS[®] HBeAg Test clinical trial were negative for HBeAg in both the VITROS[®] HBeAg Test and the FDA approved comparator method. In order to evaluate the VITROS[®] HBeAg Test performance in HBeAg reactive pediatric samples, a subset of the prospectively collected pediatric samples spiked with HBeAg was tested.

Thirty (30) individual pediatric samples, non-reactive for HBeAg, were spiked with an HBeAg positive patient sample to a target level of 2.00-4.00 S/C. Nine (30%) samples were from subjects 2 to 11 years old and 21 (70%) were from subjects 12 to 21 years old. For comparison, a base matrix material (pooled, defibrinated adult plasma, clarified, dialyzed and filtered) was spiked in the same manner into 30 individual aliquots. The matched pediatric/base matrix sets were run

in the VITROS[®] HBeAg Test in duplicate, and the average of the two replicates of each spiked pediatric sample was compared to the average of the two replicates of the associated base matrix. Results from the 30 spiked sample pairs were used to calculate the percent difference between the pediatric and the base matrix spike. The acceptance criteria were predetermined based on the assay imprecision around the cutoff plus the additional error associated with spiking individual serum samples. All 30 spiked pediatric samples met the predetermined acceptance criteria, with observed absolute percent differences in results between the pediatric sample and the base matrix ranging from 0.8% to 27.6%. The mean difference between the spiked pediatric specimens and the spiked base matrix specimens was not statistically significant at the 95% confidence level (t-test, p=0.2165). The results of this study demonstrate that the VITROS[®] HBeAg Test can effectively detect the presence of HBeAg in samples from pediatric subjects 2 to 21 years old.

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

As a diagnostic test, the VITROS[®] HBeAg 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

Multicenter clinical studies were conducted in the US. The VITROS[®] Immunodiagnostic Products HBeAg Reagent Pack, Calibrator and Controls when used on the VITROS[®] ECi/ECiQ Immunodiagnostic System performed with clinical sensitivity and specificity comparable to current commercially available FDA approved HBeAg assay.

- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in Population 1 (U.S. individuals with signs or symptoms or biochemical manifestations of hepatitis and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, or known exposure events) resulted in a positive percent agreement of 100.0% (12/12)

with a 95% confidence interval from 73.54% to 100.0% in samples with an acute HBV disease classification. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 100.0% (6/6) (confidence intervals calculated on small numbers are not statistically meaningful).

- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in Population 1 (U.S. individuals with signs or symptoms or biochemical manifestations of hepatitis and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, or known exposure events) resulted in a positive percent agreement of 88.89% (16/18) with a 95% confidence interval from 65.29% to 98.62% in samples with a chronic HBV disease classification. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 100.0% (52/52) with a 95% confidence interval from 93.15% to 100.0%.
- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in Population 2 (Indian individuals with signs or symptoms of hepatitis) resulted in a positive percent agreement of 87.50% (7/8) (confidence intervals calculated on small numbers are not meaningful) in samples with an acute HBV disease classification. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 97.83% (90/92) with a 95% confidence interval from 92.37% to 99.74%.
- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in Population 2 (Indian individuals with signs or symptoms of hepatitis) resulted in a positive percent agreement of 75.93% (41/54) with a 95% confidence interval from 62.36% to 86.51% in samples with a chronic HBV disease classification. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 84.73% (111/131) with a 95% confidence interval from 77.41% to 90.42%.
- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in individuals from Population 1 with clinically documented chronic HBV infection resulted in a positive percent agreement of 83.3% (10/12) with a 95% confidence interval from 51.6% to 97.9%. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 95.8% (23/24) with a 95% confidence interval from 78.9% to 99.9%.
- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in Russian subjects with clinically documented chronic HBV infection resulted in a positive percent agreement of 80.0% (8/10) with a 95% confidence interval from 44.4% to 97.5%. The negative percent agreement with the FDA approved comparator HBeAg test

for this group was 93.3% (28/30) with a 95% confidence interval from 77.9% to 99.2%.

- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in subjects with serologically determined chronic HBV infection resulted in a positive percent agreement of 98.68% (75/76) with a 95% confidence interval from 92.89% to 99.97%. The negative percent agreement with the FDA approved comparator HBeAg test for this group was 99.00% (199/201) with a 95% confidence interval from 96.45% to 99.88%.
- The comparison of the performance of the VITROS[®] HBeAg test to the FDA approved comparator HBeAg test in pediatric subjects in Florida at high risk for viral hepatitis resulted in a negative percent agreement of 100.0% (165/165) with a 95% confidence interval from 97.79% to 100.0%. None of the 165 pediatric samples were reactive with either the VITROS[®] HBeAg assay or the FDA approved comparator HBeAg test.
- The results of VITROS HBeAg testing with HBeAg spiked pediatric samples demonstrated that the VITROS HBeAg Test can effectively detect the presence of HBeAg in samples from pediatric subjects 2 to 21 years old.
- Seroconversion sensitivity of the VITROS[®] HBeAg test has been shown to be acceptable by testing 11 commercial seroconversion panels in comparison to a FDA approved comparator HBeAg assay.
- Analytical specificity studies evaluated the potential cross reactivity in specimens from potentially cross-reacting clinical subgroups. It has been shown that the device has no significant cross-reactivity with these potentially cross-reacting clinical subgroups, and other viral pathogens that may cause similar symptoms or are closely related to HBV. One (1) sample from the Rheumatoid Factor sub-group was reactive in the VITROS[®] HBeAg test and negative in the FDA approved comparator test.
- The VITROS[®] HBeAg test demonstrated an acceptable total variability (incorporating factors of site, lot and day) of 7.3 to 10.9%. The individual precision estimates for the different components of variance were: <5% for within day, <10% between day, < 6% between lot, < 4% between site.

The results from both the non-clinical and clinical studies indicate that the VITROS[®] HBeAg assay is effective for the *in vitro* qualitative detection of hepatitis B e antigen (HBeAg) in human adult and pediatric serum. The VITROS[®] HBeAg assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection.

C. Overall Conclusions

The data in this application support the 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[®] HBeAg assay, when compared to an FDA approved comparator, has a similar ability to detect the presence of HBeAg in serum specimens from individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection. The rate of false positivity and false negativity are within acceptable limits compared to the FDA approved comparator assay. In addition, the device has not shown any cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, this device should benefit the physician to aid in the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection.

XIII. CDRH DECISION

CDRH issued an approval order on May 11, 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).

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.

XV. REFERENCES

None