SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

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I. General Information

Device Generic Name: Antibody to Hepatitis B virus core antigen (Anti-HBc) assay and Antibody to Hepatitis B virus core antigen assay calibrator (Anti-HBc assay calibrator)

Device Trade Name: *Vitros* Immunodiagnostic Products Anti-HBc Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HBc Calibrator

Name and Address of Applicant: Ortho-Clinical Diagnostics, Inc, 100 Indigo Creek Drive, Rochester NY 14626-5101

Date of Panel Recommendation: None

PMA Number: P030024

Date of Notice of Approval to Applicant: March 4, 2004

II. Contraindications

None

III. Warnings and Precautions

The warnings and precautions can be found in the *Vitros* Immunodiagnostic Products Anti-HBc Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HBc Calibrator labeling (Attachment 1).

IV. Indications for Use

<u>*Vitros* Anti-HBc Reagent Pack</u>
For the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis B core antigen (total anti-HBc) in human adult and pediatric serum and plasma (EDTA and citrate) and neonate serum using the *Vitros* ECi Immunodiagnostic System.

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

Vitros Anti-HBc Calibrator
For use in the calibration of the *Vitros* ECi Immunodiagnostic System when used for the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis B core antigen (total anti-HBc) in human serum and plasma (EDTA and citrate).

V. Device Description

Principle of Device Methodology

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

The *Vitros* Anti-HBc assay is a competitive immunoassay, which involves the reaction of antibodies to HBc present in the specimen with recombinant hepatitis B core antigen (HBcAg) coated on the wells. Unbound material is removed by washing. Horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-HBc) is then allowed to react with the remaining exposed recombinant HBcAg on the well surface. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the *Vitros* Analyzer. The amount of HRP conjugate bound is inversely proportional to the concentration of anti-HBc present.

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

Kit Configuration and Components

For detection of anti-HBc, the *Vitros* System is comprised of the following:

- *Vitros* Immunodiagnostic Products Anti-HBc Reagent Pack (*Vitros* Anti-HBc Reagent Pack) and *Vitros* Immunodiagnostic Products Anti-HBc Calibrator (*Vitros* Anti-HBc Calibrator) together comprise the *Vitros* Anti-HBc assay.

The *Vitros* Anti-HBc Reagent Pack is composed of 3 reagents:

- Conjugate reagent [HRP-mouse monoclonal anti-HBc in buffer with mouse serum, human plasma and anti-microbial agent (Kathon)]
- Assay reagent [buffer with newborn calf serum, bovine gamma globulins and anti-microbial agent (Kathon)]
- Coated microwells [recombinant HBcAg derived from E. Coli]

The *Vitros* Anti-HBc Calibrator contains:

- Anti-HBc negative human plasma with anti-microbial agent (Kathon). The Calibrator is supplied ready for use.

In addition, the following components are required:

- *Vitros* ECi Immunodiagnostic System (*Vitros* Analyzer) is dedicated instrumentation, cleared by the FDA as an immunodiagnostic analyzer (K962919/S1), which provides automated analysis of the *Vitros* assays.
- *Vitros* Immunodiagnostic Products Signal Reagent and *Vitros* Immunodiagnostic Products Universal Wash Reagent are Common Reagents used in all *Vitros* System assays.

VI. Alternate Practices and Procedures

Determining the presence of anti-HBc in patients may be achieved by using a variety of commercially available, FDA licensed serological tests. Additionally, when test results are used in conjunction with a physician's assessment and other laboratory test results, infection with HBV can be identified.

VII. Marketing History

Below is a table describing the countries where the *Vitros* Anti-HBc Reagent Pack and Calibrator are currently available through February 2003.

Argentina	Australia
Austria	Belgium
Brazil	Canada
Chile	Colombia
Czech Republic	Denmark
France	Germany
Greece	Holland
India	Indonesia
Italy	Japan
Korea	Luxembourg
Malaysia	Norway
Panama	Philippines
Poland	Romania
Portugal	Russia
Saudi Arabia	Singapore
Slovak Republic	Slovenia
Spain	Switzerland
Taiwan	Thailand
Turkey	United Kingdom
Venezuela	

The *Vitros* Immunodiagnostic Products Anti-HBc Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HBc Calibrator have not been withdrawn from marketing for any reason relating to the safety and effectiveness of the device.

VIII. Potential Adverse Effects of the Device on Health

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

However, failure of the product to perform as indicated, or human error in use of the product may lead to a false result.

A false reactive result may be considered a patient or public health concern because false reactive results in the diagnostic setting may lead to diagnostic confusion and inappropriate therapy. This is due to the fact that both anti-HBs and anti-HBc tests may be used to determine past exposure to HBV. As appearance of anti-HBs may be delayed after HBsAg clearance, anti-HBc is sometimes the only serological marker for HBV infection. If a false reactive result is obtained for an anti-HBc test, there is a possibility that the patient would be considered to be previously exposed and therefore immune to HBV.

A false negative result in a diagnostic setting may lead to a patient with HBV going unidentified. Under these circumstances, there is a safety concern for both the patient and the public, since such individuals may be capable of transmitting HBV infection. A false negative result could also lead to in appropriate treatment in a patient with acute hepatitis.

IX. Summary of Non-Clinical Studies

Instrumentation

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

Analytical Sensitivity

The concentration at the cutoff of the *Vitros* Anti-HBc assay was confirmed by using two kit lots to assay a commercial anti-HBc (total) sensitivity serum panel that had been calibrated against serial dilutions of the Paul Ehrlich Institute anti-HBc standard (100 U/mL Anti-HBc-IgG #82). A graph of the mean *Vitros* Anti-HBc assay result versus the factored concentration of each member of the sensitivity panel was used to determine the concentration at the cutoff.

The estimated detectable concentration of anti-HBc at the cut-off (Result = 1.00 signal/cutoff (s/c)) of the *Vitros* Anti-HBc assay, estimated from the graph was 1.00 PEI Units/mL. The calculated concentration was 0.97 PEI Units/mL.

Comparison of Fresh Serum/Plasma Samples

To determine the acceptability of using the *Vitros* Anti-HBc assay for testing serum or plasma specimens, fifty fresh blood samples (25 unspiked and 25 spiked with anti-HBc to give a target result of 0.6 ± 0.4 s/c), were collected and aliquoted into a variety of serum and plasma collection tubes. Anticoagulants K_2EDTA and sodium citrate were evaluated. Testing with the *Vitros* Anti-HBc assay was conducted on the same day blood was drawn.

For matched negative samples, EDTA plasma compared with serum showed –0.6% mean difference (n=25) in anti-HBc s/c ratio and citrate plasma compared with serum showed –1.8% mean difference (n=25) in anti-HBc s/c ratio. The mean s/c ratio results for unspiked samples were equivalent by the Bonferoni test of means. All unspiked samples, with serum and plasma (EDTA and citrate) preparations, were classified correctly as negative in the *Vitros* Anti-HBc assay. The within sample precision estimates were equivalent for unspiked serum and EDTA and citrated plasma.

The s/c ratios for the anti-HBc spiked samples correlated very strongly with the s/c ratios of serum. EDTA and citrate plasma had equivalent minimum and maximum s/c ratios. Although the serum samples had higher estimates of within sample precision than citrate plasma, differences in precision were mostly (92.75%) accounted for by differences in mean s/c ratio results.

The above results indicate that serum and plasma (EDTA and citrate) should be suitable for use in the *Vitros* Anti-HBc assay.

Comparison of Stability of Serum/Plasma Samples

Twenty fresh blood samples (10 unspiked and 10 spiked with anti-HBc to give a target result of 0.6 ± 0.4 s/c), were collected and aliquoted into a variety of serum and plasma collection tubes. Anticoagulants K_2EDTA and sodium citrate were evaluated. Testing with the *Vitros* Anti-HBc assay was conducted on the same day blood was drawn, and again after 5 and 7 days storage at 2 – 8 °C (36°- 46 °F) and after 28 days at -20 °C (-4 °F).

None of the storage conditions tested had clinically significant effects on reactive samples. All anti-HBc spiked samples were qualitatively classified correctly as reactive, regardless of the storage condition. When analyzed statistically, there were five statistically significant positive slopes over time for anti-HBc spiked samples stored at 2 – 8 °C (36 - 46 °F). However, these statistically significant differences over time did not change the qualitative classification of the specimens; therefore they are not clinically significant.

These data show that storage of serum or plasma (EDTA and citrate) samples for up to 5 days at 2 – 8 °C (36 - 46 °F), or 28 days at -20°C (-4°F) should not have a significant effect on the test results with the *Vitros* Anti-HBc assay.

Potentially Cross-reacting Subgroups

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

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

The specificity of the *Vitros* Anti-HBc assay was evaluated further by testing anti-HBc spiked and unspiked samples with an additional spike of *Staphylococcus aureus, Escherichia coli* or *Pseudomonas aeruginosa.*

Of the samples that were tested none of the anti-HBc unspiked (negative) samples were found to be false reactive and none of the anti-HBc spiked samples were observed to be false negative in the *Vitros* Anti-HBc assay.

Interfering Substances

The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 10 blood donors. The results (test results at each level of interferent) demonstrate that hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL) and triolein (up to 3000 mg/dL), should have no effect on result classification.

Samples spiked with anti-HBc to give a target result of 0.7 – 0.9 s/c were observed to remain reactive at all levels tested with each potential interferent. Similarly no interference was observed in samples not spiked with anti-HBc (negative).

Stability

Vitros Anti-HBc Reagent Packs, Calibrator and Controls that were subjected to a period of simulated transport to mimic effects of shipment were tested at various time points up to 26 weeks after storage at 2 – 8 °C (36 – 46 °F). All results obtained were within acceptability limits, and overall no trends were evident.

In addition, a commercially obtained performance panel was tested using transported, stored materials at week 0 and week 26. Materials stored for 26 weeks yielded results that indicated no change in the qualitative classification of the samples from the classifications obtained at the initial time point.

These data supports the storage of the *Vitros* Anti-HBc Reagent Pack, Calibrator and Controls for 26 weeks at 2 – 8 °C (36 – 46 °F).

Open On-Board Storage for the *Vitros* Anti-HBc Reagent Pack

Vitros Anti-HBc Reagent Packs that were subjected to a period of simulated transport to mimic effects of shipment were opened and placed in an environmental chamber (4 – 8 °C, ≤ 40% humidity) for a period of 8 weeks to simulate the storage on board the *Vitros* Analyzer. These Reagent Packs were tested at various time points within the 8 week time period. In addition, a single transported, opened Reagent Pack from each kit lot was removed from the chamber on 6 different occasions, and brought to room temperature over the 8 week period to simulate typical customer usage. Results of testing were within acceptability limits and overall no trend was observed between Reagent Packs stored at 2 – 8 °C (36 – 46 °F) and freshly opened, and Reagent Packs stored opened on board for 8 weeks. These data supports the on board storage of Reagent Packs for up to 8 weeks.

Open Off-Board Storage for *Vitros* Anti-HBc Calibrators

Vitros Anti-HBc Calibrators that were subjected to a period of simulated transport to mimic effects of shipment were opened, pooled, sub-aliquoted and stored at 2 - 8 °C (36 – 46 °F) and -20 °C (-4 °F) for 13 weeks. Results of testing these calibrators at various time points up to 13 weeks indicated no observable trends and met all acceptance criteria.

The data supports the storage of the calibrators at 2 – 8 °C (36 – 46 °F) and -20°C (-4°F) after opening for up to 13 weeks (with no more than 1 freeze-thaw cycle).

Vitros Universal Wash Reagent Study

Vitros Anti-HBc Reagent Packs, calibrators, and controls that were subjected to a period of simulated transport to mimic the effects of shipment were tested with 3 lots of *Vitros* Universal Wash Reagent at weeks 0 and 26 to determine the effect of aged *Vitros* Universal Wash Reagent.

The data show that the performance of the *Vitros* Anti-HBc assay is acceptable when used with *Vitros* Universal Wash Reagent that is either fresh or up to 26 weeks old.

Vitros Signal Reagent Study

Vitros Anti-HBc Reagent Packs, calibrators, and controls that were subjected to a period of simulated transport to mimic the effects of shipment were tested with 3 lots of *Vitros* Signal Reagent to determine the effect of aged *Vitros* Signal Reagent.

The data show that the performance of the *Vitros* Anti-HBc assay is acceptable when used with *Vitros* Signal Reagent that is either fresh or 6 months old.

Temperature Stressing Study (-20 °C / -4 °F)

Vitros Anti-HBc assay Reagent Packs and calibrators were subjected to 2 freeze/thaw cycles and the performance compared with Reagent Packs and calibrators stored at 2 - 8 °C (36 – 46 °F). Although freezing and thawing the *Vitros* Anti-HBc assay had no adverse effect on calibration quality parameters or control results, as a precaution it is recommended that the *Vitros* Anti-HBc assay is not frozen.

Temperature Stressing Study (30 °C and 37 °C / 86 °F and 99 °F)

Vitros Anti-HBc Reagent Packs and calibrators were subjected to 5 days at 30 °C (86 °F) or 1 day at 37 °C (99 °F) and the performance compared with Reagent Packs and calibrators stored at 2 – 8 °C (36 – 46 °F).

Exposing *Vitros* Anti-HBc Reagent Packs and calibrators to a temperature of 30 °C (86 °F) for 5 days or 37 °C (99 °F) for 1 day had no adverse effect on calibration quality parameters or control results. Exposure of the Reagent Packs or calibrators up to these temperatures for the times stated should not significantly compromise the performance of the *Vitros* Anti-HBc assay.

Microbiology

Vitros Anti-HBc reagents are formulated with anti-microbial agents (Bronidox and Kathon) that provide protection against adventitious contamination by microorganisms. Evaluation of the microbial load of each reagent (Assay Reagent, Conjugate Reagent and Calibrator) post-dispensing and at 26 weeks demonstrated that the total aerobic count is generally on the order of $\leq$10 CFU/mL. In addition, the levels of preservative in each reagent were determined over a period of at least 26 weeks. Results for the Assay Reagent, Conjugate Reagent and Calibrator demonstrated that the preservative concentrations were above the minimum inhibitory concentration throughout 26 weeks of testing.

A study conducted according to US Pharmacopoeia (USP) 23/NF 18, general chapter 51, assessed the ability of the reagents to withstand or control microbial contamination. Results indicate that the preservative systems for Assay Reagent and Calibrator met the requirements of the USP 23 at 0 and 26 weeks. The Conjugate Reagent met the USP 23 requirement at week 0 for all challenge microorganisms and at week 26 for all but *carnobacterium divergens*.

Precision and Reproducibility

Precision was evaluated on a different *Vitros* ECi Immunodiagnostic System at three external sites, using one reagent pack and calibrator kit lot. At least two replicates each of a three member panel were assayed on a single occasion per day on 20 different days. The data shown in the table below were rounded following all calculations.

Clinical Site	Mean *Vitros* aHBc S/C (Ratio)	Within Day *		Between Day †		Total ‡		No. Obs.	No. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Site 1	3.52	0.091	2.6	0.093	2.6	0.130	3.7	40	20
	0.34	0.026	7.7	0.019	5.6	0.032	9.5	40	20
	0.95	0.030	3.2	0.060	6.4	0.067	7.1	40	20
Site 2	3.50	0.064	1.8	0.039	1.1	0.075	2.1	40	20
	0.40	0.037	9.2	0.056	14.1	0.067	16.9	40	20
	1.10	0.082	7.4	0.062	5.6	0.103	9.3	40	20
Site 3	3.43	0.071	2.1	0.089	2.6	0.113	3.3	40	20
	0.31	0.014	4.3	0.023	7.4	0.027	8.6	40	20
	0.90	0.027	3.0	0.025	2.8	0.037	4.1	40	20

* Within Day: Variability of the assay performance from replicate to replicate.
† Between Days: Variability of the assay performance from day to day.
‡ Total: Variability of the assay performance combining the effects of within day and between days.

Reproducibility was evaluated incorporating between site and between lot variations. The study was performed at three external sites using three reagent lots. At least three replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates (CV (%)) were derived from a variance component analysis. The data shown in the table below were rounded following all calculations.

Mean *Vitros* Anti-HBc S/C (Ratio)	Between Site *		Between Lot †		Total ‡		No. Obs.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
3.21	0.000	0.0	0.071	2.2	0.159	5.0	162
1.19	0.121	10.2	0.047	4.0	0.155	13.0	162
1.10	0.121	11.0	0.059	5.4	0.157	14.3	162
0.25	0.090	35.9	0.017	7.0	0.102	40.7	162

* Between site: Variability of the assay performance from site to site.
† Between lot: Variability of the assay performance from lot to lot, calculated using data across all sites.
‡ Total: Variability of the assay incorporating factors of site, lot and day.

Calibration Interval

The performance of the *Vitros* Anti-HBc assay within and beyond one instrument calibration interval (28 days) was evaluated at three sites by testing a three member sera panel with one kit lot. One panel member was close to the *Vitros* Anti-HBc assay cut-off. 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. Two replicates of each panel member were assayed per day at each clinical site. Appropriate instrument calibration was performed and verified on Day 0 of the study, and the testing was performed for a total of 20 study days over a 28-day period.

Least squares regression analyses were performed within site and across sites. For analyses within site, although the slopes were statistically significant for two panel members in at least one site, the changes in s/c ratios over the entire testing period were either not clinically relevant, i.e., did not change the qualitative result interpretation, or were too small to have any clinical implications. For analyses across sites, the mean slope was not statistically significant for any of the panel members.

The *Vitros* Anti-HBc assay demonstrated adequate performance throughout the entire calibration interval, and continued to perform successfully two days beyond the expiration of calibration, as per the study design.

Seroconversion Panels

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

Days to Reactive Anti-HBc Result					
Panel ID	Reference Anti-HBc Assay		*Vitros* Anti-HBc Assay		Difference in Days to Anti-HBc Reactive Result
	- *	+ **	- *	+ **	Reference - *Vitros*
6278	26	33	33	37	-4
6281	36	41	36	41	0
PHM935A	50	66	50	66	0
RP009	13	29	13	29	0
RP016	24	56	24	56	0
RP017	43	65	43	65	0

* Post bleed day of last nonreactive result, usually denotes previous bleed from first reactive.

** Post bleed day of first reactive result.

Based on the results of the testing, the *Vitros* Anti-HBc assay demonstrated agreement with the time of anti-HBc comparable to the reference anti-HBc assay.

Cord Blood Testing

A total of 20 cord blood samples were tested in the *Vitros* Anti-HBc assay.

In testing the cord blood samples, 1 out of 20 samples was found to give a repeat reactive result in the *Vitros* Anti-HBc assay. This repeat reactive sample was also repeat reactive in the reference method.

Duplicate aliquots from 10 cord blood samples were spiked with either anti-HBc positive or negative plasma. Recovery of anti-HBc from serum and cord blood was assessed by calculating the percent shift of results [(negative spike-reactive spike)/negative spike]. The mean percent shift for serum was 75.8%. The mean percent shift for cord blood was 76.2%. It appears that anti-HBc is recovered from cord blood to the same extent as in serum.

The above information shows that cord blood should be an acceptable sample type for use with the *Vitros* Anti-HBc assay. Due to the low sample numbers and a single matrix being tested OCD has stated in the indications for use statement that serum may only be used and placed a warning in the Interpretations of Results stating that low level results should not be repeated. The warning implies that another specimen should be obtained and tested.

X. Summary of Clinical Studies

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

The HBV disease classification for each subject was determined by a single point serological assessment using a hepatitis marker profile consisting of reference assays (previously licensed or approved by the FDA) for the detection of HBsAg, HBeAg, anti-HBc, anti-HBc IgM, anti-HBe, and anti-HBs (quantitative). The reference assays' procedures were adhered to during the clinical laboratory study.

The subjects in Population I were Caucasian (24.9%), African American (44.1%), Hispanic (22.4%) and Asian (3.7%), with the remaining 4.9% represented by other ethnic groups. The group was 52.4% male and 47.6% female, and ranged in age from 5 to 89 years. Testing of these specimens with the *Vitros* Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (37.0%), Port Jefferson, NY (34.9%) and Minneapolis MN, (28.1%). Agreement of the *Vitros* Anti-HBc assay was assessed relative to the reference anti-HBc assay and HBV disease classification using serum samples from the 1691 subjects in Population I.

The subjects in Population II were Asian Indian (100%). The group was 73.0% male and 27.0% female, and ranged in age from 18 to 90 years. Testing of these specimens with the *Vitros* Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (33.0%), Minneapolis MN, (32.4%) and Los Angeles, CA (34.6%). Agreement of the *Vitros* Anti-HBc assay was assessed relative to a reference anti-HBc assay and HBV disease classification using serum samples from the 315 subjects in Population II.

Results by Specimen Classification

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

HBV Reference Marker Profiles and HBV Disease Classification

Reference HBsAg[1,2]	Reference HBeAg	Reference IgM aHBc	Reference Total aHBc	Reference aHBe	Reference aHBs ≥10 mIU/mL	HBV Disease Classification
+	+	+	+	+	–	Acute
+	+	+	+	–	–	Acute
+	–	+	+	+	+	Acute
+	–	+	+	+	–	Acute
+	–	+	+	–	–	Acute
+	–	–	–	–	–	Acute
+	+	–	+	+	–	Chronic
+	+	–	+	–	+	Chronic
+	+	–	+	–	–	Chronic
+	–	–	+	+	+	Chronic
+	–	–	+	+	–	Chronic
+	–	–	+	–	–	Chronic
–	–	+	+	+	+	Early Recovery
–	–	+	+	+	–	Early Recovery
–	–	+	+	–	+	Early Recovery
–	–	+	+	–	–	Early Recovery
–	–	–	+	+	–	Early Recovery
–	–	–	+	+	+	Recovery
–	–	–	+	–	+	Recovered
–	–	–	+	–	–	Recovered
–	–	–	–	–	+	HBV Vaccine Response
–	–	–	–	–	–	Not Previously Infected with HBV
+	+	–	–	+	+	Uninterpretable
+	–	–	–	–	+	Uninterpretable
–	+	–	+	–	–	Uninterpretable
–	+	–	–	–	+	Uninterpretable
–	+	–	–	–	–	Uninterpretable
–	–	+	–	–	–	Uninterpretable

1. Positive = Reference HBsAg assay reactive and confirmed by neutralization.
2. Negative = Reference HBsAg assay negative or not confirmed by neutralization.

Comparison of Results

The table below compares the *Vitros* Anti-HBc results with the reference anti-HBc results by specimen classification for the subjects in Population I.

Comparison of *Vitros* Anti-HBc Results with Reference Anti-HBc Results by HBV Disease Classification - Population I (N=1691)

	Reference Anti-HBc Result				
	Reactive		Negative		
HBV Disease Classification	*Vitros* Anti-HBc Result		*Vitros* Anti-HBc Result		
	Reactive	Negative *	Reactive	Negative	Total
Acute	8	0	0	9	17
Chronic	40	3	0	0	43
Early Recovery	46	1	0	0	47
Recovery	138	0	0	0	138
Recovered	168	28	0	0	196
HBV Vaccine Response	0	0	0	169	169
Not Previously Infected with HBV	0	0	5 **	1069	1074
Uninterpretable	0	1	0	6	7
Overall	400	33	5	1253	1691

* These samples were tested with a second FDA approved anti-HBc assay with the following results:
Chronic: 2/3 negative,
Early recovery: 1/1 indeterminate,
Recovered: 18/28 negative; 1/28 indeterminate,
Uninterpretable: 1/1 negative,
Overall: 23/33 (69.7%) negative or indeterminate.

** These samples were tested with a second FDA approved anti-HBc assay with the following results: 2/5 (40%) positive.

The table below compares the *Vitros* Anti-HBc results with the reference anti-HBc results by specimen classification for the subjects in Population II.

Comparison of *Vitros* Anti-HBc Results With Reference Anti-HBc Results by HBV Disease Classification – Population II (N=315)

	Reference Anti-HBc Result				
	Reactive		Negative		
HBV Disease Classification	*Vitros* Anti-HBc Result		*Vitros* Anti-HBc Result		
	Reactive	Negative *	Reactive	Negative	Total
Acute	86	2	0	16	104
Chronic	184	1	0	0	185
Early Recovery	1	0	0	0	1
Recovery	0	0	0	0	0
Recovered	2	1	0	0	3
HBV Vaccine Response	0	0	0	3	3
Not Previously Infected with HBV	0	0	0	17	17
Uninterpretable	0	0	0	2	2
Overall	273	4	0	38	315

* Zero of three samples were negative with a second FDA approved anti-HBc assay. One sample (Chronic) was not tested (insufficient volume.)

Percent Agreement

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

Positive and Negative Percent Agreement between the *Vitros* Anti-HBc and Reference Anti-HBc Assays in Population I

HBV Disease Classification	Positive Percent Agreement (%)	95% Exact Confidence Interval	Negative Percent Agreement (%)	95% Exact Confidence Interval
Overall	400/433 (92.38%)	89.46, 94.70	1253/1258 (99.60%)	99.07, 99.87
Acute	8/8 (100%)	63.06, 100	9/9 (100%)	66.37, 100
Chronic	40/43 (93.02%)	80.94, 98.54	0/0 (N/A)	N/A
Early Recovery	46/47 (97.87%)	88.71, 99.95	0/0 (N/A)	N/A
Recovery	138/138 (100%)	97.36, 100	0/0 (N/A)	N/A
Recovered	168/196 (85.71%)	80.02, 90.29	0/0 (N/A)	N/A
HBV Vaccine Response	0/0 (N/A)	N/A	169/169 (100%)	97.84, 100
Not Previously Infected with HBV	0/0 (N/A)	N/A	1069/1074 (99.53%)	98.92, 99.85
Uninterpretable	0/1 (0%)	N/A	6/6 (100%)	54.07, 100

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

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

Positive and negative percent agreement between the *Vitros* Anti-HBc assay and the reference anti-HBc assay were also calculated for subjects in Population II (N=315) with various HBV disease classifications, and for the overall study population. The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals.

Positive and Negative Percent Agreement between the *Vitros* Anti-HBc and Reference Anti-HBc Assay in Population II

HBV Disease Classification	Positive Percent Agreement (%)	95% Exact Confidence Interval	Negative Percent Agreement (%)	95% Exact Confidence Interval
Overall	273/277 (98.56%)	96.34, 99.61	38/38 (100%)	90.75, 100
Acute	86/88 (97.73%)	92.03, 99.72	16/16 (100%)	79.41, 100
Chronic	184/185 (99.46%)	97.03, 99.99	0/0 (N/A)	N/A
Early Recovery	1/1 (100%)	2.5, 100	0/0 (N/A)	N/A
Recovered	2/3 (66.67%)	9.43, 99.16	0/0 (N/A)	N/A
HBV Vaccine Response	0/0 (N/A)	N/A	3/3 (100%)	29.24, 100
Not Previously Infected with HBV	0/0 (N/A)	N/A	17/17 (100%)	80.49, 100
Uninterpretable	0/0 (N/A)	N/A	2/2 (100%)	15.81, 100

The positive percent agreement with the reference anti-HBc assay was determined by dividing the number of reactive *Vitros* Anti-HBc results by the total number of subjects reactive with the reference anti-HBc assay. As a result of this study, the overall positive percent agreement of the *Vitros* Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 98.56% (273/277, with a 95% exact confidence interval of 96.34, 99.61).

The negative percent agreement with the reference anti-HBc assay was determined by dividing the number of negative *Vitros* Anti-HBc results by the total number of subjects negative with the reference anti-HBc assay. As a result of this study, the overall negative percent agreement of the *Vitros* Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 100% (38/38, with a 95% exact confidence interval of 90.75, 100).

Percent Agreement of the *Vitros* Anti-HBc Assay With Clinical Status for Subjects With Clinically Diagnosed Acute or Chronic HBV Infection

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

Overall Clinical Performance of the *Vitros* Anti-HBc Assay in Samples From Subjects With Clinically Documented Acute or Chronic HBV Infection

HBV Infection	Number of Samples	Number (%) of *Vitros* Anti-HBc Reactive Samples	95% Exact Confidence Interval
Acute	8	8 (100.0)	63.06, 100
Chronic	76	75 (98.7)	92.89, 99.97
Total	84	83 (98.8)	93.54, 99.97

For the information contained above0, percent agreement for the *Vitros* Anti-HBc Assay is generally greater than 90% (lower 95% confidence interval). When percent agreement is less than 90% (95% CI lower bounds) it is believed that the lower percent agreement may be due to the low numbers of specimens tested in each disease classification or differences between the two assays, e.g., antigen or detector antibody differences. The above information shows that the *Vitros* Anti-HBc Assay has similar performance compared to a reference anti-HBc assay in the HBV disease categories listed. It is believed that with the above performance the *Vitros* Anti-HBc Assay will furnish useful and meaningful results when used as indicated.

Clinical Performance of the *Vitros* Anti-HBc Assay in Pre-Vaccination Samples

Serum samples obtained from 41 individuals immediately prior to HBV vaccination were tested with the *Vitros* and reference anti-HBc assays. The results are shown below for both assays.

***Vitros* and Reference Anti-HBc Results in Pre-Vaccination Samples (N=41)**

Test Result	Reference Anti-HBc Assay	*Vitros* Anti-HBc Assay
Initially Negative	37	41
Initially Reactive	4	NA
Repeatedly Reactive	0	NA
Total Negative Results	41	41

NA = Not applicable

Potentially Cross-Reacting Subgroups

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

Comparison of *Vitros* and Reference Anti-HBc Results and HBV Disease Classification among Anti-HAV IgM Reactive Study Subjects - Population I (N=7)

	Reference Anti-HBc Result				
	Reactive		Negative		
HBV Disease	*Vitros* Anti-HBc Result		*Vitros* Anti-HBc Result		
Classification	Reactive	Negative	Reactive	Negative	Total
Acute	0	0	0	0	0
Chronic	0	0	0	0	0
Early Recovery	0	0	0	0	0
Recovery	0	0	0	0	0
Recovered	2	0	0	0	2
HBV Vaccine Response	0	0	0	0	0
Not Previously Infected with HBV	0	0	0	5	5
Uninterpretable	0	0	0	0	0
Overall	2	0	0	5	7

Comparison of *Vitros* and Reference Anti-HBc Results and HBV Disease Classification among Anti-HCV Reactive Study Subjects - Population I (N=353)

	Reference Anti-HBc Result				
	Reactive		Negative		
	Vitros Anti-HBc Result		*Vitros* Anti-HBc Result		
HBV Disease Classification	Reactive	Negative	Reactive	Negative	Total
Acute	1	0	0	3	4
Chronic	8	1	0	0	9
Early Recovery	25	0	0	0	25
Recovery	43	0	0	0	43
Recovered	92	8	0	0	100
HBV Vaccine Response	0	0	0	22	22
Not Previously Infected with HBV	0	0	1	147	148
Uninterpretable	0	1	0	1	2
Overall	169	10	1	173	353

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

Comparison of *Vitros* and Reference Anti-HBc Results and HBV Disease Classification among Anti-HAV IgM Reactive Study Subjects - Population II (N=29)

HBV Disease Classification	Reference Anti-HBc Result				
	Reactive		Negative		
	Vitros Anti-HBc Result		*Vitros* Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	Total
Acute	10	1	0	7	18
Chronic	0	1	0	0	1
Early Recovery	0	0	0	0	0
Recovery	0	0	0	0	0
Recovered	0	0	0	0	0
HBV Vaccine Response	0	0	0	3	3
Not Previously Infected with HBV	0	0	0	6	6
Uninterpretable	0	0	0	1	1
Overall	10	2	0	17	29

Comparison of *Vitros* and Reference Anti-HBc Results and HBV Disease Classification among Anti-HCV Reactive Study Subjects - Population II (N=90)

HBV Disease Classification	Reference Anti-HBc Result				
	Reactive		Negative		
	Vitros Anti-HBc Result		*Vitros* Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	Total
Acute	58	0	0	0	58
Chronic	32	0	0	0	32
Early Recovery	0	0	0	0	0
Recovery	0	0	0	0	0
Recovered	0	0	0	0	0
HBV Vaccine Response	0	0	0	0	0
Not Previously Infected with HBV	0	0	0	0	0
Uninterpretable	0	0	0	0	0
Overall	90	0	0	0	90

XI. Conclusions Drawn From Studies

These data demonstrate acceptable performance is obtained with the *Vitros* Anti-HBc assay when testing specimens collected in serum and plasma (EDTA and citrate).

The *Vitros* Anti-HBc Reagent Pack and Calibrator can be stored for up to 26 weeks at 2 - 8 °C (36 – 46 °F). After opening, the Reagent Pack can be stored on-board the *Vitros* Analyzer (4 – 8 °C (39 – 46 °F), ≤40% relative humidity) for up to 8 weeks, and the Calibrator stored for up to 13 weeks at 2 – 8 °C (36 – 46 °F) or -20 °C (-4 °F) (with no more than one freeze-thaw cycle).

The preservative systems that the *Vitros* Anti-HBc assay reagents are formulated with have been shown to meet USP 23 requirements at 26 weeks for Assay Reagent and Calibrator. The Conjugate Reagent meets the USP 23 requirements at 26 weeks with the exception of *carnobacterium divergens.*

The *Vitros* Anti-HBc assay demonstrated adequate precision estimates for within day and between day for each site as well as across all sites, and between replicate, between day, between site and between lots when these variables were introduced.

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

The *Vitros* Anti-HBc assay agrees with a reference anti-HBc assay in five out of six seroconversion panels. One of the six panels was detected as being repeatedly reactive one bleed later than determined by the reference assay.

Cord blood samples did not interfere with the *Vitros* Anti-HBc assay, and anti-HBc is recovered from cord blood to the same extent as in serum.

The results of the clinical laboratory studies provide reasonable assurance the *Vitros* Anti-HBc assay, when used according to the provided instructions for use, is safe and effective for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection. The presence of anti-HBc may be used as an aid in the determination of exposure to HBV infection for individuals prior to HBV vaccination.

XII. Panel Recommendation

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 Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CDRH Decision

FDA issued an approval order on March 4, 2004.

The applicant's manufacturing facility was inspected on June 14, 2002 and found to be in compliance with the Quality Systems Regulation (21 CFR 820).

XIV. Approval Specifications

Directions for use: See the labeling.

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

Postapproval Requirements and Restrictions: See approval order.