SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

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

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

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

Date of Panel Recommendation: None

Premarket Approval Application Number: P030026

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

II. Indications for Use

Reagent Pack

For the *in vitro* qualitative detection of IgM antibody to hepatitis B core antigen (anti-HBc IgM) in human adult and pediatric serum and plasma (heparin, 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.

Calibrator

For use in the calibration of the *Vitros* ECi Immunodiagnostic System when used for the *in vitro* qualitative detection of IgM antibody to hepatitis B virus core antigen (anti-HBc IgM) in human adult and pediatric serum and plasma (EDTA, heparin and citrate) and neonate serum using *Vitros* Anti-HBc IgM Reagent Packs.

III. Contraindications: None known

IV. Warnings and Precautions

The warnings and precautions can be found in the *Vitros* Anti-IgM Assay Reagent Pack and *Vitros* Anti-IgM Assay Calibrator labeling.

The *Vitros* Anti-HBc IgM Calibrator has been validated for use only on the *Vitros* System with V*itros* Immunodiagnostic Products anti-HBc IgM Reagent Packs. Refer to the *Vitros* Anti-HBc IgM Reagent Pack instructions for use for further details.

V. Device Description

Kit Configuration and Components

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

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

The *Vitros* Anti-HBc IgM Reagent Pack is composed of three components:

- Biotinylated Antibody Reagent
  Biotin-mouse monoclonal anti-human IgM antibody in buffer with bovine serum albumin and anti-microbial agent (Kathon).

- Conjugate Reagent
  Horse Radish Peroxidase - mouse monoclonal anti-HBc antibody with recombinant HBc antigen derived from *E. coli* in buffer with bovine serum albumin and anti-microbial agent (Kathon).

- Streptavidin coated microwells (52 wells per pack).

The *Vitros* Anti-HBc IgM Calibrator contains:

- Anti-HBc IgM positive human defibrinated plasma (inactivated) 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 random access instrumentation, 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.

- *Vitros* Immunodiagnostic Products High Sample Diluent B – a buffer with bovine serum albumin and antimicrobial agent (Bronidox-L). High Sample Diluent B is a Common Reagent used with select *Vitros* System assays.

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

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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 touch screen monitor.

The *Vitros* Anti-HBc IgM assay is performed using the *Vitros* Anti-HBc IgM Reagent Pack and the *Vitros* Anti-HBc IgM Calibrator on the *Vitros* Immunodiagnostic System. The assay uses an antibody class capture technique which involves dilution of the sample and the simultaneous reaction of IgM in the diluted sample with biotinylated mouse monoclonal anti-human IgM antibody. The immune complex is captured by streptavidin on the wells; unbound materials are removed by washing. Horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBc antibody which has been complexed with recombinant HBc antigen (conjugate) is then captured by anti-HBc specific IgM bound to the wells. Unbound material 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 *Vitros* Analyzer reads the light signals. The amount of HRP conjugate bound is directly proportional to the concentration of anti-HBc IgM present in the sample.

The *Vitros* Anti-HBc IgM Calibrator allows calibration of the *Vitros* Anti-HBc IgM assay setting a boundary for interpretation of positive and negative results. 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 cutoff value are encoded on the 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 cutoff 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.

VI. **Alternate Practices and Procedures**

Determining the presence of anti-HBc IgM in patients may be achieved by using a variety of commercially available, FDA licensed or approved 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

The following table lists the countries where the *Vitros* Immunodiagnostic Products Anti-HBc IgM Assay may be marketed as of March 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 IgM Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HBc IgM 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 or negative result may be considered a patient or public health concern because false results in the diagnostic setting may lead to diagnostic confusion and inappropriate therapy. IgM class antibodies against HBc are detected in high concentrations soon after infection with HBV and are an indicative marker for acute hepatitis. The detection of anti-HBc IgM is also used in the differential diagnosis of hepatitis B from other forms of viral hepatitis. A false negative result may lead to a diagnosis of autoimmune hepatitis (AH), and administration of immunosuppressive drugs potentially harmful to the patient. A false

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positive result might delay the diagnosis of AH, which requires early aggressive therapy. The risk of unnecessary liver biopsy might also be increased with erroneous anti-HBc IgM results.

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 IgM Reagent Pack, functioned as described and have appropriate safeguards.

Comparison of Fresh Serum/Plasma Samples

To determine the acceptability of using the *Vitros* Anti-HBc IgM assay for testing serum or plasma specimens, fifty fresh blood samples (25 unspiked and 25 spiked with anti-HBc IgM to give a target result of 2.0 ± 1.0 signal/cut-off (s/c))[1], were collected by syringe and dispensed serum collection tubes, and collection tubes containing sodium heparin, K_2 EDTA, and sodium citrate anticoagulants. Testing with the *Vitros* Anti-HBc IgM assay was conducted on the same day blood was drawn.

For matched anti-HBc IgM spiked samples, EDTA plasma compared with serum showed 0.5% mean difference (n=25) in anti-HBc IgM s/c ratio, heparin plasma compared with serum showed 1.6% mean difference (n=25) in anti-HBc IgM s/c ratio and citrate plasma compared with serum showed –7.7% mean difference (n=25) in anti-HBc IgM s/c ratio. The mean s/c ratios for EDTA and heparin plasma were not statistically different from the mean s/c ratio for serum. The mean s/c ratio (n=25) for citrate plasma was observed to be statistically different from the mean s/c ratio for serum. Part of this difference may be attributed to the dilution of the blood into the citrate buffer. Though there was a statistically significant difference, there was no clinical difference. A statement is included in the Limitation of Procedure section of the labeling cautioning the user concerning low positive or high negative results from citrated plasma. All of the anti-HBc IgM spiked samples tested maintained reactivity in the *Vitros* Anti-HBc IgM assay regardless of the sample type preparation. The within sample precision estimates were equivalent for anti-HBc IgM spiked serum and EDTA, heparin and citrate plasma.

All unspiked samples, with serum and plasma (EDTA, heparin, and citrate) preparations, were classified correctly as negative in the *Vitros* Anti-HBc IgM assay. Although the EDTA plasma samples had higher estimates of within sample precision than the other matrices, differences in precision were mostly (99.79%) accounted for by differences in mean s/c ratio results.

The results indicate that serum and plasma (EDTA, heparin, and citrate) are suitable for use in

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[1] Where ≥ 1.1 s/c is reactive for anti-HBc IgM and < 0.90 s/c is nonreactive for anti-HBc IgM. The range ≥ 0.9 to ≤ 1.1 s/co denotes an indeterminate result and the specimen requires duplicate retesting.

the *Vitros* Anti-HBc IgM assay, if the user is aware that there will be a slight dilutional factor when sodium citrate is used.

Comparison of Stability of Serum/Plasma Samples

The stability of serum and plasma samples for use in the *Vitros* Anti-HBc IgM assay was investigated. Twenty fresh blood samples (10 unspiked and 10 spiked with Anti-HBc IgM to give a target result of 2.0 ± 1.0 s/c), were collected by syringe and dispensed into serum and plasma (heparin, sodium citrate, and K_2 EDTA) collection tubes. Testing with the *Vitros* Anti-HBc IgM 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).

Statistical analysis of the data resulted in significantly different positive slopes for negative samples stored at 2-8 °C (36-46 °F) and negative slopes for positive samples stored 2-8 °C (36-46 °F). Though statistically significant, all samples were classified correctly.

For samples stored at -20 °C (-4 °F), statistically significant differences were seen for negative heparin plasma and serum samples over the 28 day time period. Similar analysis for the anti-HBc IgM spiked samples resulted in statistically significant mean decreases among all sample types. However, none of the observed differences over time changed the classification of the specimens.

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

Potentially Cross-reacting Subgroups

The specificity of the *Vitros* Anti-HBc IgM assay was evaluated by testing 244 samples from 16 potentially cross-reacting sub-groups. Patient samples from the following sub-groups were tested: HAV, HEV, 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, heterophilic antibodies and anti-HBc Positive/anti-HBc IgM Negative.

None of the 244 samples from16 sub-groups were found to be anti-HBc IgM reactive with the comparator method (Abbott) or the *Vitros* Anti-HBc assay.

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

None of the anti-HBc IgM unspiked (negative) samples were found to be false reactive and none of the anti-HBc IgM spiked samples were observed to be false negative in the presence or absence of the addition of potential cross reacting microorganisms in the *Vitros* Anti-HBc IgM assay.

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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 samples spiked with hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL), and triolein (up to 3000 mg/dL), should cause no misclassification of qualitative results.

Anti-HBc IgM spiked samples were tested near the cut-off (reactive (s/c) ≥ 1.00). No change in qualitative interpretation was observed for spiked samples at all levels tested with each potential interferent. Similarly no interference was observed in samples not spiked with anti-HBc IgM (negative), with anti-HBc IgM values remaining < 1.00 s/c.

Stability

Vitros Anti-HBc IgM Reagent Packs, Calibrators 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 result interpretation of the samples from the qualitative results obtained at the initial time point.

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

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

Vitros Anti-HBc IgM 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, $\leq$ 40% humidity) for a period of 8 weeks to simulate the storage on board the *Vitros* Analyzer. These Reagent Packs were tested at various timepoints within the 8 week time period. In addition, a single transported, opened Reagent Pack from each Kit Lot was removed from the chamber on six 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 the *Vitros* Anti-HBc IgM Reagent Packs for up to 8 weeks.

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

Vitros Anti-HBc IgM 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 timepoints 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 one freeze-thaw cycle).

Vitros Universal Wash Reagent Study

Vitros Anti-HBc IgM 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 months 0 and 6 to determine the effect of aged *Vitros* Universal Wash Reagent.

The data presented in this report show that the performance of the *Vitros* Anti-HBc IgM assay is acceptable when used with *Vitros* Universal Wash Reagent which is either fresh or up to 6 months old.

Vitros Signal Reagent Study

Vitros Anti-HBc IgM Reagent Packs, calibrators, and controls that were subjected to a period of simulated transport to mimic the effects of shipment were tested with 4 lots of *Vitros* Signal Reagent to determine the effect of aged *Vitros* Signal Reagent.

The data presented in this report show that the performance of the *Vitros* Anti-HBc IgM assay is acceptable when used with *Vitros* Signal Reagent which is either fresh or up to 6 months old.

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

Vitros Anti-HBc IgM 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 IgM 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 does not appear to significantly compromise the performance of the *Vitros* Anti-HBc IgM assay.

Microbiology

Vitros Anti-HBc IgM reagents are formulated with an anti-microbial agent (Kathon) that provides protection against adventitious contamination by microorganisms. 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, conjugate reagent and high sample diluent B met the requirements of the USP 23 at 0 and 26 weeks.

Precision

Precision was evaluated on a different *Vitros* ECi Immunodiagnostic System at each of 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 were rounded following all calculations.

Clinical Site	Mean *Vitros* Anti-HBc IgM S/C (Ratio)	Within day *		Between day		Total ‡		No. Obs.	No. Days
		SD	CV (%)	SD†	CV (%)	SD	CV (%)		
Site 1	0.01	0.001	11.0	0.001	10.5	0.001	15.2	40	20
	1.95	0.021	1.1	0.033	1.7	0.039	2.0	40	20
	0.98	0.021	2.2	0.012	1.3	0.025	2.5	40	20
Site 2	0.01	0.001	16.1	0.000	0.0	0.001	16.1	40	20
	1.89	0.023	1.2	0.047	2.5	0.052	2.8	40	20
	0.96	0.014	1.4	0.025	2.6	0.028	2.9	40	20
Site 3	0.01	0.001	8.6	0.000	6.0	0.001	10.5	40	20
	1.95	0.021	1.1	0.032	1.6	0.038	2.0	40	20
	0.97	0.015	1.6	0.017	1.8	0.023	2.4	40	20

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

Reproducibility

Precision was further 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 were rounded following all calculations.

Mean *Vitros* Anti-HBc IgM S/C (Ratio)	Between Site *		Between Lot †		Total ‡		No. Obs.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
2.49	0.076	3.0	0.097	3.9	0.135	5.4	162
1.06	0.031	3.0	0.025	2.3	0.047	4.5	162
0.97	0.027	2.8	0.016	1.7	0.040	4.1	162
0.23	0.008	3.4	0.008	3.8	0.013	5.7	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.

Instrument Calibration Interval

The performance of the *Vitros* Anti-HBc IgM 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 IgM assay cutoff. Additional testing was performed on Day 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 calibration was performed and verified on Day 1 of the study, and the testing was performed for a total of 20 study days over a 28-day period.

The least squares regression analyses were performed within site and across sites. For analyses within site, although the slopes were statistically significant for each of the panel members at 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 so small that they would not have any clinical implications.

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

Seroconversion Panels

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

Anti-HBc IgM Seroconversion Panel Study - Summary Results

Panel ID	Days to Reactive Anti-HBc IgM Result				
	Reference Anti-HBc IgM Assay		*Vitros* Anti-HBc IgM Assay		Difference in Days to Anti-HBc IgM Reactive Result
	- [*]	+ [**]	- [*]	+ [**]	Reference - *Vitros*
6278	33	37	37	41	-4
6281	41	43	41	43	0
PHM935A	50	66	66	68	-2
RP009	36	43	36	43	0
RP016	56	59	56	59	0
RP017	76	78	76	78	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 IgM assay demonstrated agreement with the time of anti-HBc IgM comparable to the reference anti-HBc IgM assay.

Cord Blood Testing

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

In testing the cord blood samples, 0 out of 20 samples were found to give a repeat reactive result in the *Vitros* Anti-HBc IgM assay. Results of the reference method were also all negative.

Recovery of anti-HBc IgM from serum and cord blood was assessed by calculating the difference in result for each specimen when spiked with anti-HBc IgM reactive plasma or anti-HBc IgM negative plasma. The mean difference for serum specimens was 1.98 s/c. The mean difference for cord blood specimens was 2.04 s/c. It appears that anti-HBc IgM is recovered from the cord blood matrix to a similar extent as serum.

The above information shows that cord blood should be an acceptable sample type for use with the *Vitros* Anti-HBc IgM 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 IgM 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 IgM 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 IgM 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 IgM assay was assessed relative to the reference anti-HBc IgM assay and HBV disease classification using serum samples from the 1691 subjects in Population I.

The subjects in Population II were Asian Indian (100.0%). The group was 73.0% male and 27.0% female, and ranged in age from 18 to 90 years. Testing of specimens with the *Vitros* Anti-HBc IgM 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 IgM assay was assessed relative to the reference anti-HBc IgM assay and HBV disease classification using serum samples from the 315 subjects in Population II.

XI. 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 IgM clinical study.

HBV Reference Marker Profiles and HBV Disease Classification

Reference HBsAg[1,2]	Reference HBeAg	Reference IgM aHBc	Reference Total aHBc	Reference aHBc	Reference aHBs $\geq$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.

XII. Comparison of Results

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

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	VITROS Anti-HBc IgM Result		VITROS Anti-HBc IgM Result		
	Reactive	Negative *	Reactive **	Negative	Total
Acute	8	0	0	9	17
Chronic	0	0	2	41	43
Early Recovery	4	6	1	36	47
Recovery	0	0	0	138	138
Recovered	0	0	1	195	196
HBV Vaccine Response	0	0	0	169	169
Not Previously Infected with HBV	0	0	0	1074	1074
Uninterpretable	0	1	0	6	7
Overall	12	7	4	1668	1691

* These samples were tested with a second FDA approved anti-HBc IgM assay with the following results:

Early recovery: 2/6 negative

Uninterpretable: 1/1 negative

Overall: 3/7 (42.9%) negative agreement with VITROS

** These samples were tested with a second FDA approved anti-HBc IgM assay with the following results:

Chronic: 2/2 positive

Early Recovery: 1/1 positive

Recovered: 1/1 positive

Overall: 4/4 (100%) positive agreement with VITROS

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

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	VITROS Anti-HBc IgM Result		VITROS Anti-HBc IgM Result		
	Reactive	Negative *	Reactive	Negative	Total
Acute	69	19	0	16	104
Chronic	0	0	0	185	185
Early Recovery	1	0	0	0	1
Recovery	0	0	0	0	0
Recovered	0	0	0	3	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	70	19	0	226	315

* These samples were tested with a second FDA approved anti-HBc IgM assay with the following results:

Acute: 8/19 negative; 3/19 indeterminate

Overall: 11/19 (57.9%) negative or indeterminate

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XIII. Percent Agreement

Positive and negative percent agreement between the *Vitros* Anti-HBc IgM assay and the reference anti-HBc IgM 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 IgM and Reference Anti-HBc IgM Assays in Population I

HBV Disease Classification	Positive Percent Agreement (%)	95% Exact Confidence Interval	Negative Percent Agreement (%)	95% Exact Confidence Interval
Overall	12/19 (63.16%)	38.36, 83.71	1668/1672 (99.76%)	99.39, 99.93
Acute	8/8 (100%)	63.06, 100	9/9 (100%)	66.37, 100
Chronic	0/0 (N/A)	N/A	41/43 (95.35%)	84.19, 99.43
Early Recovery	4/10 (40%)	12.16, 73.76	36/37 (97.3%)	85.84, 99.93
Recovery	0/0 (N/A)	N/A	138/138 (100%)	97.36, 100
Recovered	0/0 (N/A)	N/A	195/196 (99.49%)	97.19, 99.99
HBV Vaccine Response	0/0 (N/A)	N/A	169/169 (100%)	97.84, 100
Not Previously Infected with HBV	0/0 (N/A)	NA	1074/1074 (100%)	99.66, 100
Uninterpretable	0/1 (0%)	N/A	6/6 (100%)	54.07, 100

In Population I, overall positive percent agreement of the *Vitros* Anti-HBc IgM assay with the reference anti-HBc IgM assay was estimated to be 63.16% (12/19, with a 95% exact confidence interval of 38.36% to 83.71%). Overall negative percent agreement of the *Vitros* Anti-HBc IgM assay with the reference anti-HBc IgM assay in Population I was estimated to be 99.76% (1668/1672, with a 95% exact confidence interval of 99.39, 99.93).

Positive and negative percent agreement between the *Vitros* Anti-HBc IgM assay and the reference anti-HBc IgM 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 IgM and Reference Anti-HBc IgM Assays in Population II

HBV Disease Classification	Positive Percent Agreement (%)	95% Exact Confidence Interval	Negative Percent Agreement (%)	95% Exact Confidence Interval
Overall	70/89 (78.65%)	68.69 - 86.63	226/226 (100%)	98.38, 100
Acute	69/88 (78.41%)	68.35 - 86.47	16/16 (100%)	79.41, 100
Chronic	0/0 (N/A)	N/A	185/185 (100%)	98.03, 100
Early Recovery	1/1 (100%)	2.5 - 100	0/0 (N/A)	N/A
Recovered	0/0 (N/A)	N/A	3/3 (100%)	29.24, 100
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 overall positive percent agreement of the *Vitros* Anti-HBc IgM assay with the reference anti-HBc IgM assay in Population II was estimated to be 78.65% (70/89, with a 95% exact confidence interval of 68.69% to 86.63%). Overall negative percent agreement of the *Vitros* Anti-HBc IgM assay with the reference anti-HBc IgM assay in Population II was estimated to be 100.0% (226/226, with a 95% exact confidence interval of 98.38, 100.0).

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

The performance of the *Vitros* Anti-HBc IgM 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 IgM assay in samples from subjects with documented acute or chronic HBV infection.

Overall Clinical Performance of the Vitros Anti-HBc IgM Assay in Samples from Subjects with Clinically Documented Acute or Chronic HBV Infection

HBV Infection	Number of Samples	Number (%) of *Vitros* Anti-HBc IgM Reactive Samples	95% Exact Confidence Interval	Number (%) of Reference Anti-HBc IgM Reactive Samples	95% Exact Confidence Interval
Acute	8	8 (100)	63.1, 100	8 (100)	63.1, 100
Chronic	76	11 (14.5)	7.5, 24.4	30 (39.5)	28.4, 51.3

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

XV. 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 IgM results with reference anti-HBc IgM results according to the HBV disease classifications assigned to the study subjects.

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	Vitros Anti-HBc IgM Result		*Vitros* Anti-HBc IgM Result		
	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	0	0	0	2	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	0	0	0	7	7

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	VITROS Anti-HBc IgM Result		VITROS Anti-HBc IgM Result		
	Reactive	Negative	Reactive	Negative	Total
Acute	1	0	0	3	4
Chronic	0	0	0	9	9
Early Recovery	1	3	0	21	25
Recovery	0	0	0	43	43
Recovered	0	0	1	99	100
HBV Vaccine Response	0	0	0	22	22
Not Previously Infected with HBV	0	0	0	148	148
Uninterpretable	0	0	0	2	2
Overall	2	3	1	347	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 IgM results with reference anti-HBc IgM results according to the HBV disease classifications assigned to the study subjects.

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	Vitros Anti-HBc IgM Result		*Vitros* Anti-HBc IgM Result		
	Reactive	Negative	Reactive	Negative	Total
Acute	3	8	0	7	18
Chronic	0	0	0	1	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	3	8	0	18	29

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

HBV Disease Classification	Reference Anti-HBc IgM Result				
	Reactive		Negative		
	VITROS Anti-HBc IgM Result		VITROS Anti-HBc IgM Result		
	Reactive	Negative	Reactive	Negative	Total
Acute	45	13	0	0	58
Chronic	0	0	0	32	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	45	13	0	32	90

XVI. Conclusions Drawn from Studies

The *Vitros* Anti-HBc IgM 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, $\leq$ 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 with which the *Vitros* Anti-HBc IgM assay reagents are formulated have been shown to meet USP 23 requirements at 26 weeks for the assay Reagent, Conjugate Reagent, Calibrator, and High Sample Diluent B.

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

The *Vitros* Anti-HBc IgM assay agreed with a reference assay for four out of six seroconversion panels in determining the point at which the panel went from negative to repeatedly reactive. Two seroconversion panels were determined by the *Vitros* Anti-HBc IgM assay to become repeatedly reactive one bleed after a reference assay determined the panel to be repeatedly reactive. For one panel member, both the reference and the *Vitros* Anti-HBc IgM assay identified the point where the panel went from being negative to repeatedly reactive, but the *Vitros* Anti-HBc IgM assay identified that panel to then become repeatedly negative again before the reference assay showed consistent non-reactive results.

The *Vitros* Anti-HBc IgM Assay does not detect interference with cord blood samples (from newborn subjects). Both the reference and the *Vitros* Anti-HBc IgM assay detected 0 out of twenty reactive samples. Cord blood spiked with anti-HBc IgM positive serum reacts similarly to spiked adult serum.

The results of the clinical laboratory studies provide reasonable assurance the *Vitros* Anti-HBc IgM 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.

XVII. 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.

XVIII. 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).

XIX. 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.