Summary of Safety and Effectiveness

1. General Information

1.1. Device Generic Name

Antibody to Hepatitis B Surface Antigen (Anti-HBs) assay

1.2. Device Trade Name

Vitros Immunodiagnostic Products Anti-HBs Reagent Pack
Vitros Immunodiagnostic Products Anti-HBs Calibrators

1.3. Name and Address of Applicant

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

1.4. PMA Number

P000014

1.5. Date of Panel Recommendation

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

1.6. Date of Notice of Approval to Applicant

September 29, 2000

2. Indications for Use

2.1. *Vitros* Immunodiagnostic Products Anti-HBs Reagent Pack (*Vitros* Anti-HBs Assay):

For the qualitative in vitro determination of total antibody to hepatitis B surface antigen (anti-HBs) in human serum using the *Vitros* ECi Immunodiagnostic System.

Assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection for individuals prior to or following HBV vaccination, or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is unknown.

Refer to device labeling for warnings and precautions related to this device.

2.2. *Vitros* Immunodiagnostic Products Anti-HBs Calibrators:

For use in the calibration of the *Vitros* Immunodiagnostic System for the qualitative *in vitro* determination of total antibody to hepatitis B surface antigen (anti-HBs) in human serum using *Vitros* Anti-HBs Reagent Packs.

The *Vitros* Anti-HBs Calibrators have been validated for use only on the *Vitros* System with the *Vitros* Immunodiagnostic Products Anti-HBs Reagent Pack. Refer to the *Vitros* Anti-HBs Reagent Pack instructions for use for further details.

The *Vitros* Anti-HBs Calibrators are traceable to the World Health Organization (WHO) First International Reference Preparation for Antibody to HBsAg (1977).

3. Device Description

3.1. Principle of Device Methodology

3.1.1. *Vitros* Immunodiagnostic Products Anti-HBs Reagent Pack

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

The *Vitros* Anti-HBs assay incorporates an immunometric technique involving the reaction of anti-HBs present in the sample with hepatitis B surface antigen (HBsAg) coated on the wells. Following removal of unbound material by washing, anti-HBs is detected by a horseradish peroxidase (HRP)-labeled HBsAg (conjugate). Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescence 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 indicative of the concentration of anti-HBs present.

3.1.2. *Vitros* Immunodiagnostic Products Anti-HBs Calibrators

Calibration is lot specific; reagent packs and calibrators are linked by lot number. A Master Calibration is established for each new reagent lot by performing multiple assays on a number of *Vitros* Systems. Values for the linked lot of calibrators are determined from the Master Calibration. These values, and the data that enables a *Vitros* System to reconstruct the Master Calibration, are encoded on the lot calibration card.

Scanning the lot calibration card loads the encoded data onto the *Vitros* System. When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration is assessed against a range of quality parameters, if acceptable it is stored for use with any reagent pack of that lot. The quality of calibration cannot be completely described by a single parameter. The calibration report must be used in conjunction with verifier values or control ranges to determine the validity of the calibration. Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.

The calibrators establish the assay cutoff for the *Vitros* Anti-HBs Reagent Pack. This cutoff is representative of approximately 10 mIU of anti-HBs/mL of serum. Individuals whose specimens are reactive at this level or higher have been determined to be immune from infection with HBV.[1]

3.2. Kit Configuration and Components

For detection of anti-HBs, the *Vitros* system is comprised of the following:

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

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

- Conjugate reagent [HRP labeled HBsAg (heat inactivated) subtypes ad and ay, 0.33 µg/mL, in buffer with human serum, bovine serum albumin and antimicrobial agent (Kathon)].

- Assay reagent with antimicrobial agent (Kathon).

- Coated microwells [heat inactivated HBsAg subtypes ad and ay, coated at 40 P.E.I. Units*/well]. (* Paul-Ehrlich-Institute HBsAg Reference Material)

The *Vitros* Anti-HBs Calibrators contain:

- Human recalcified plasma with antimicrobial agent (Bronidox). Calibrators are supplied ready for use. The Calibrators are traceable to the WHO First International Reference Preparation for Antibody to HBsAg (1977).

3.3. In addition, the following components are required:

- *Vitros* ECi Immunodiagnostic System (*Vitros* Analyzer)– dedicated instrumentation.

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[1] Recommendations of the Immunization Practices Advisory Committee Update on Hepatitis B Prevention. MMWR. 1987: 36(23): 353-366.

- *Vitros* Immunodiagnostic Products Signal Reagent and *Vitros* Immunodiagnostic Products Universal Wash Reagent.

4. Contraindications, Warnings and Precautions

For in vitro diagnostic use only.

Warnings and precautions for users of the *Vitros* Anti-HBs Reagent Pack and *Vitros* Anti-HBs Calibrators are stated in the respective product labeling.

5. Alternate Practices and Procedures

Determining the presence of anti-HBs in patients may be achieved by using a variety of commercially available, FDA licensed, serological tests. Additionally, when test results are used in combination with a physician's assessment and biochemical tests, susceptibility to HBV can be excluded.

6. Marketing History

Sales of the *Vitros* Immunodiagnostic Products Anti-HBs Reagent Pack and *Vitros* Immunodiagnostic Products Calibrators in foreign markets through October 1999 are presented in the following table.

Country
Argentina
Australia
Belgium
Canada
Chile
Denmark
France
Germany
Holland
India
Italy
Norway
Portugal
Singapore
South Africa
Spain
United Kingdom

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

7. Potential Adverse Effects of the Device on Health

Failure of the product to perform as indicated, or human error in use of the product, may lead to a false result. A false nonreactive result cannot be considered a patient or public health concern, as the patient would either unnecessarily receive a vaccine, vaccine booster, hyperimmune globulin, or be considered not to have recovered from an acute HBV infection when they have.

A false reactive assay result may be a patient or public health concern due to the fact that a patient would be considered to be previously exposed and therefore immune to HBV or that the patient was successfully vaccinated. In this case, the risk is that the patient would not receive a vaccine, vaccine booster, hyperimmune globulin, and would be at higher risk of infection if exposed to HBV. Once exposed, the risk of this patient spreading infection to uninfected or non-immune members of the community increases.

8. Summary of Non-Clinical Studies

8.1. Additional 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-HBs Reagent Pack, functioned as described and had appropriate safeguards.

8.2. Analytical Sensitivity

The concentration at the cut-off (result = 1.00) of the *Vitros* Anti-HBs assay was defined by using three kit lots to assay a series of dilutions having known concentrations of the WHO 1st International Reference Preparation. A linear regression of the mean *Vitros* Anti-HBs assay result (n=18) versus the calculated concentration of each dilution was used to determine the concentration of anti-HBs at the assay's cut-off.

The concentration at the cut-off of the *Vitros* Anti-HBs assay, estimated from the linear regression, was 10.97 mIU anti-HBs/mL (95%C.I. = 10.77 to 11.18 mIU anti-HBs/mL). The dilution series with the WHO 1st International Reference showed that a rise from 0% to 100% frequency occurred between 8.1 and 12.3 mIU/mL, inclusive. A 0% frequency means that the detection rate of anti-HBs reactive samples was too small to measure, while a 100% frequency means that the failure to detect anti-HBs reactive samples was too small to measure.

8.3. Potentially Cross-Reacting Subgroups

The specificity of the *Vitros* Anti-HBs assay was evaluated by testing 209 samples from 16 potentially cross-reacting sub-groups. Patient samples from the following sub-groups were tested: HAV, HCV, HEV, non-viral liver disease, autoimmune disease (rheumatoid arthritis and systemic lupus erythrematosis), CMV, EBV, HSV (type not specified), B19 virus (parvovirus B19) infection, rubella, syphilis, toxoplasmosis, HIV 1/2 antibody reactive, HTLV 1/2 antibody reactive and recent influenza vaccine recipients. In addition, samples from 47 blood donors were tested in the study.

Of these 16 sub-groups 197 out of 209 samples were observed to be nonreactive. One anti-rubella IgM specimen was reactive and eleven specimen results were found to be indeterminate in the *Vitros* Anti-HBs assay. All blood donor samples tested were observed to be nonreactive.

The following table shows a testing result summary:

Clinical Category	Number Samples Tested	*Vitros* Anti-HBs assay Result < 0.50	*Vitros* Anti-HBs assay Result ≥ 0.50 and < 1.20	*Vitros* Anti-HBs assay result ≥ 1.20
Hepatitis A Infection (HAV)	10	10	0	0
Hepatitis C Infection (HCV)	10**	10	0	0
Hepatitis E Infection (HEV)	4	4	0	0
Non-viral Liver Disease	50	46	4††	0
Autoimmune Diseases (Rheumatoid Arthritis / Systemic Lupus Erythematosus)	49	49	0	0
Cytomegalovirus (CMV)	5	5	0	0
Epstein-Barr Virus (EBV)	10	9	1	0
Herpes Simplex Virus (HSV)	10	10	0	0
Parvovirus B19 Infection	5	5	0	0
Rubella	10	6	3	1‡‡
Syphilis	10	9	1	0
Toxoplasmosis	8	8	0	0
Human Immunodeficiency Virus (HIV 1/2)	10	10	0	0
Human T-cell Lymphotropic Virus (HTLV 1/2)	10	9	1	0
Recent Influenza Vaccine Recipients	8	7	1	0
Total Samples Tested	209	197	11	1

**Two of these samples were EIA repeatedly reactive and RIBA positive.
††Two of these samples were from the same patient and were initially ≥ 1.20.
‡‡Sample coagulated - retest not possible

8.4. Interfering Substances

The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 10 blood donors as recommended by the National Committee for Clinical Laboratory Standards' Protocol EP7-P.[2] Blood from each donor was divided into two aliquots. One aliquot was spiked with anti-HBs to a target *Vitros* result of 1.5 – 2.0 (≥ 10 mIU/mL anti-HBs), and the other spiked with an equal volume of anti-HBs nonreactive plasma. The anti-HBs reactive and nonreactive pools from each donor were separated into 6 aliquots for the bilirubin and triolein test substances. Hemolyzed samples were prepared using a freeze-thaw method. A dilution series was prepared to provide both anti-HBs reactive and nonreactive sample pools. Anti-HBs was added (as described above), to the hemolyzed and non-hemolyzed pools to give a target result of 1.5 – 2.0 in the final sample. Autologous clear plasma was used to provide a hemoglobin nonreactive control.

[2] NCCLS. Interference Testing in Clinical Chemistry; Proposed Guideline. NCCLS document EP7-P (ISBN 1-56238-020-6). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1986.

The results were as follows:

- Four of 10 (4/10) samples spiked to contain approximately 10 mIU/mL of anti-HBs in the hemoglobin study tested indeterminate for all levels of hemoglobin tested, 0 - 500 mg/dL. Since the control level (0 mg/dL) for each of these samples resulted in indeterminate results it is considered that hemoglobin was not a cause of the indeterminate results. One of 10 (1/10) samples in the hemoglobin, anti-HBs nonreactive dilution series had repeat results that would be classified as indeterminate at 300 mg hemoglobin/dL.

- One of 10 (1/10) samples in the bilirubin, anti-HBs spiked dilution series, at 20 mg/dL bilirubin, had an initial result of indeterminate. Of the 10 samples in the bilirubin, anti-HBs nonreactive dilution series one sample was indeterminate in five of twelve results, 0-20 mg bilirubin/dL.

- One of 10 samples in the triolein, anti-HBs spiked series at the 1000 mg/mL level had an initial result of indeterminate.

8.5. Stability

Vitros Anti-HBs Reagent Packs, Calibrators, and Controls were subjected to a period of simulated transport. To simulate the transport conditions, Reagent Packs, Calibrators and *Vitros* Anti-HBs 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 the transported materials were compared to results obtained from non-transported materials to verify that in-house quality control results were not affected.

Using three Master Lots of transported materials; four runs were performed at weeks 0, 4, 9, 13, 18, 22, 26, 36, 45 and 52. Each run contained single determinations of the Calibrators, in-house quality controls, and *Vitros* Anti-HBs Controls. Results from each time point were used to assess and establish assay performance over time.

Performance Panel samples were assayed at weeks 0, 26 and 52. Four runs consisting of single determinations of each panel member, Calibrators, in-house quality controls, and *Vitros* Anti-HBs Controls were performed using three Master Lots of transported material. Results from controls were assessed against their acceptability limits in order to ensure the validity of each run.

Based on means for the two specimens closest to the assay's cutoff it was theoretically calculated that over the 52 week expiration period a specimen at the assay's cutoff (1.00 = approximately 10 mIU anti-HBs/mL) will vary from 0.82 to 1.18. For a specimen at the lower equivocal result range limit, 0.50, its result will vary from 0.47 to 0.53. Therefore, there appears to be little variation in the reagents over the claimed expiry period. Probable reactive specimens with values near the assay's cutoff would become equivocal, requiring testing to verify the reported result. Probable nonreactive specimens near the lower equivocal zone range would be reported as nonreactive.

The information presented appears to support the claimed 52-week expiration period when reagents are shipped at conditions not exceeding 20 °C for 2 days.

Vitros Anti-HBs Reagent Pack and Calibrators were temperature stressed at 30°C for 5 days and 37 °C for 1 day. The reagent packs and calibrators were then used to test four *Vitros* Anti-HBs Controls and five in-house controls. This testing demonstrated that inadvertent exposure of the Reagent Packs or Calibrators up to temperatures of 30°C or 37°C for the times stated would not significantly compromise the performance of the *Vitros* Anti-HBs assay.

8.6. Open On-Board Storage for the *Vitros* Anti-HBs Reagent Pack

Vitros Anti-HBs Reagent Packs were opened and placed in an environmental chamber (4-8 °C, ≤ 40% relative 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 period. In addition, an opened Reagent Pack from each master lot was removed from the chamber and brought to room temperature on six different occasions over the 8 week period to simulate typical customer usage.

Results of testing were within acceptability limits and overall, no trend was observed between freshly opened Reagent Packs stored at 2-8 °C and Reagent Packs stored opened on-board for 8 weeks. These data supports the on-board storage of Reagent Packs for up to 8 weeks.

8.7. Open Off-Board Storage for *Vitros* Anti-HBs Calibrators

Vitros Anti-HBs Calibrators that were subjected to a period of simulated transport to mimic effects of shipment (see Section 8.5) were opened, pooled, sub-aliquoted and stored at 2-8 °C and at -20 °C for a period of 13 weeks. Results of testing these Calibrators at time points up to 13 weeks indicated no observable trends and met all acceptance criteria.

The data support the storage of the Calibrators at 2-8 °C and at -20 °C after opening for up to 13 weeks (with no more than one freeze-thaw cycle).

8.8. Microbiology

Vitros Anti-HBs reagents are formulated with anti-microbial agents (Kathon and Bronidox) that provide protection against adventitious contamination by microorganisms. Evaluation of the microbial load of each reagent (Assay Reagent, Conjugate Reagent and 3 levels of Calibrators) post-dispensing and at 61 weeks (post-expiry) demonstrated that the total aerobic count is generally in the order of ≤10CFU/mL. In addition, the levels of preservative in each reagent (Assay Reagent, Conjugate Reagent and 3 levels of Calibrators) were determined over a period of 30 weeks of storage. Results demonstrated that the preservative concentrations in each reagent were above the minimum inhibitory concentration throughout the 30 weeks.

A study conducted according to US Pharmacopoeia (USP) 23/NF 18, general chapter 51, assessed the ability of the reagents to withstand or control microbial contamination. Results indicated that the preservative systems for each reagent met the requirements of the USP 23 at 45 weeks for Assay Reagent, 31 weeks for Conjugate Reagent, and 27 weeks for Calibrator 1 (which is representative of all Calibrator levels) based on the date of manufacture for each reagent.

8.9. Precision

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

	Anti-HBs *Vitros* Mean assay result	Within Day*		Between Day†		Total‡			
		SD	CV (%)	SD	CV (%)	SD	CV (%)	No. Observ.	No. Days
Site 1	1.06	0.058	5.5	0.083	7.9	0.102	9.6	40	20
	2.59	0.036	1.4	0.073	2.8	0.081	3.1	40	20
	8.81	0.168	1.9	0.268	3.0	0.316	3.6	40	20
	34.5	0.396	1.1	0.630	1.8	0.745	2.2	40	20
Site 2	1.20	0.057	4.8	0.037	3.0	0.068	5.6	38	19
	2.65	0.029	1.1	0.077	2.9	0.082	3.1	38	19
	8.51	0.123	1.4	0.091	1.1	0.153	1.8	40	19
	32.2	0.115	0.4	0.745	2.3	0.754	2.3	40	19
Site 3	1.13	0.045	4.0	0.056	4.9	0.072	6.3	40	20
	2.55	0.028	1.1	0.062	2.4	0.068	2.7	41	20
	8.55	0.058	0.7	0.115	1.3	0.129	1.5	41	20
	33.6	0.173	0.5	0.270	0.8	0.321	1.0	41	20

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

8.10. Reproducibility

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

Mean *Vitros* anti-HBs assay result	Between Site*		Between Lot†		Total‡		No. Observ.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
0.62	0.036	5.8	0.000	0.0	0.075	12.0	274
0.95	0.036	3.8	0.107	11.3	0.127	13.4	271
1.05	0.048	4.6	0.015	1.5	0.085	8.1	270
4.17	0.166	4.0	0.499	12.0	0.550	13.2	271

***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: total variability of the assay performance incorporating factors of site, lot, and day

8.11. Calibration Interval

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

Least square regression analyses were performed within site and across sites. As a general trend, the *Vitros* Anti-HBs assay results were slightly decreased over the testing period within one calibration interval. For the analyses within site, although the slope was statistically significant for some combinations of site and panel member, the changes in results over the testing period were small enough that they would appear not to have clinical implications, i.e., a specimen that should be a low value reactive would test as indeterminate, never nonreactive. For analyses across sites, the slope was not statistically significant for the panel member approximating 10 mIU anti-HBs/mL, clinically the most important panel member. Although the slope was statistically significant for some other panel members, the changes in results over the entire testing period were so small that they appear not to be considered clinically significant.

8.12. Seroconversion Panels

Four commercially available seroconversion panels collected as 4% citrated plasma were tested. *Vitros* Anti-HBs assay results using fresh citrated plasma samples have been shown to be approximately 20% lower than corresponding serum samples. These four panels were tested because they were the only available panels that included bleeds from individuals at the time of HBV disease onset through seroconversion. All Day 0 specimens contained no detectable anti-HBs by the *Vitros* anti-HBs or an FDA licensed reference assay.

- For three panels, detection of anti-HBs by the *Vitros* Anti-HBs assay (above assay's cutoff level) occurred at Days 54, 87, and 201. For these panels the reference assay showed similar results.

- One panel was nonreactive for anti-HBs by the *Vitros* Anti-HBs assay through Day 188 (last panel member), but was reactive by the reference anti-HBs assay on Day 181.

Overall, the *Vitros* Anti-HBs assay and reference anti-HBs assay demonstrated similar performance for the detection of the appearance of anti-HBs after infection with HBV.

9. Summary of Clinical Studies

A multi-center prospective study was conducted to evaluate the clinical performance of the *Vitros* Anti-HBs assay in individuals with signs or symptoms of hepatitis. Also included were asymptomatic individuals at high risk of HBV infection due to lifestyle, behavior, occupation, or known exposure events. Specimens were prospectively collected from sites located in Miami, FL (37%), Dallas, TX (39%), Chicago, IL (23%), and New York, NY (1%). The group was Caucasian (28%), African American (43%) and Hispanic (23%) with the remaining 6% represented by three or more ethnic groups. The group was 55% male and 45% female and ranged in age from 5 to 96

years. The HBV disease classification for each subject was determined by serological assessment using resultant hepatitis marker profiles obtained from reference assays' results (previously licensed or approved by the FDA) for the detection of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), total antibody to hepatitis B core antigen (anti-HBc), IgM antibody to hepatitis B core antigen (anti-HBc IgM), total antibody to hepatitis B e antigen (anti-HBe), and total antibody to hepatitis B surface antigen (anti-HBs) (quantitative). All reference assays used were from a single manufacturer. The reference assays' procedures were adhered to during the clinical laboratory study. Testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (37%), Dallas, TX (39%), and New York, NY (24%). Agreement of the *Vitros* Anti-HBs assay results were assessed relative to the reference anti-HBs results and the specimen classification using serum samples from a total of 1761 subjects (fourteen individuals were excluded from the complete study set (1775) due to these samples lacking full reference HBV serological marker results).

Approximately 61% (1078/1775) of the prospective subjects participating in the *Vitros* Anti-HBs clinical study reported no recent or current signs or symptoms of hepatitis. Of the 1078 asymptomatic individuals, 24.8% were enrolled in Miami, FL, 53.3% were enrolled in Dallas, TX, 21.4% were enrolled in Chicago, IL, and 0.5% were enrolled in New York, NY. The group was Caucasian (31%), African American (45%) and Hispanic (19%) with the remaining 5% represented by three or more ethnic groups. The group was 56% male and 44% female and ranged in age from 5 to 96 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The *Vitros* Anti-HBs assay was reactive in 27% of the individuals in this group. The percent *Vitros* Anti-HBs reactive results observed in the asymptomatic population at each site was 25% at Miami, FL, 32% at Dallas, TX, 15% at Chicago, IL, and 60% at New York, NY. The table below summarizes the percent *Vitros* Anti-HBs reactive, nonreactive, and indeterminate results by gender and age range.

Age Range	Gender	*Vitros* Anti-HBs Result						Total
		+		-		I		
		n	Percent	n	Percent	n	Percent	
0 - 9	F	0	NA	0	NA	0	NA	0
	M	1	100	0	NA	0	NA	1
10 - 19	F	6	46	7	54	0	NA	13
	M	1	10	9	90	0	NA	10
20 - 29	F	32	37	53	61	2	2	87
	M	21	25	62	74	1	1	84
30 - 39	F	34	29	81	69	3	2	118
	M	35	19	133	74	12	7	180
40 - 49	F	39	33	72	61	7	6	118
	M	60	31	123	64	9	5	192
50 - 59	F	16	22	52	71	5	7	73
	M	21	26	59	74	0	NA	80
60 - 69	F	9	21	33	77	1	2	43
	M	4	11	31	84	2	5	37
70 - 79	F	2	12	15	88	0	NA	17
	M	5	31	11	69	0	NA	16
80 - 89	F	2	50	2	50	0	NA	4
	M	0	NA	1	100	0	NA	1
90 - 100	F	0	NA	0	NA	0	NA	0
	M	0	NA	1	100	0	NA	1
	Total	288		745		42		1075*

*Age not reported for three subjects.

The complete study data set (asymptomatic and symptomatic for HBV infection) was analyzed following the assignment of specimen classification based upon the reactive (+)/nonreactive (-) patterns for six HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process. There were 22 unique reference marker patterns observed in the *Vitros* Anti-HBs clinical study (see classification summary table below).

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

The table below compares the *Vitros* Anti-HBs results with the anti-HBs reference assay for each specimen classification. The data in the table are representative of the number of specimens in each result category. Due to a modification of the *Vitros* Anti-HBs assay's indeterminate range in the clinical study, specimens that had *Vitros* Anti-HBs indeterminate results ≥ 0.80 and < 1.20 were retested in duplicate. Specimens with indeterminate results ≥ 0.50 and < 0.80 were not retested.

HBV Classification	Anti-HBs Reference Result								Total
	-				+				
	Vitros Anti-HBs Result				*Vitros* Anti-HBs Result				
	-	+	I*	I#	-	+	I*	I#	
Acute	21	0	0	0	0	0	0	0	21
Chronic	39	0	1	1	1	1	0	1	44
Early Recovery	32	5	1	10	0	8	0	1	57
Recovery	0	0	0	0	2	138	3	2	145
Recovered	70	3	6	4	2	134	10	5	234
Uninterpretable	7	0	0	1	0	3	0	0	11
HBV Vaccine Response	0	0	0	0	2	210	5	2	219
Not Previously Infected	993	10	7	20	0	0	0	0	1030
Total	1162	18	15	36	7	494	18	11	1761

*Indeterminate result following repeat testing.
#Indeterminate result without repeat testing.

The table below summarizes the percent agreement between the *Vitros* Anti-HBs assay and the anti-HBs reference assay for each specimen classification, and provides the upper and lower 95% exact confidence bounds. Agreement was calculated as follows, indeterminate *Vitros* anti-HBs results were included in the calculations:

$$\text{Positive percent agreement} = \left(\frac{\text{number of Vitros aHBs reactive in agreement with reference aHBs}}{\text{Total number reference aHBs reactive}}\right)\times 100$$

$$\text{Negative percent agreement} = \left(\frac{\text{number of Vitros aHBs nonreactive in agreement with reference aHBs}}{\text{Total number reference aHBs nonreactive}}\right)\times 100$$

$$\text{Overall percent agreement} = \left(\frac{\text{number of Vitros aHBs results in agreement with reference aHBs}}{\text{Total number reference aHBs reactive and nonreative}}\right)\times 100$$

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Overall	93.2 (494/530)	90.7 to 95.2	94.4 (1162/1231)	93.0 to 95.6
Acute	NA	NA	100 (21/21)	83.9 to 100
Chronic	33.3 (1/3)	0.8 to 90.6	95.1 (39/41)	83.5 to 99.4
Early Recovery	88.9 (8/9)	51.8 to 99.7	66.7 (32/48)	51.6 to 79.6
Recovery	95.2 (138/145)	90.3 to 98.0	NA	NA
Recovered	88.7 (134/151)	82.6 to 93.3	84.3 (70/83)	74.7 to 91.4
Uninterpretable	100 (3/3)	29.2 to 100	87.5 (7/8)	47.4 to 99.7
HBV Vaccine Response	95.9 (210/219)	92.3 to 98.1	NA	NA
Not Previously Infected	NA	NA	96.4 (993/1030)	95.1 to 97.5

A study was conducted to evaluate 187 retrospective serum samples from individuals who had received a full course of injections of either SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® HBV vaccine. The *Vitros* Anti-HBs assay was reactive in 98.4% (184/187) of the post-vaccination samples. This is comparable to the reactivity of 97.9% (183/187) demonstrated by the reference anti-HBs method (calibrated to furnish a reactive result of ≥ 10 mIU/mL). There were no observable differences in results among subjects receiving either the Merck & Co. or SmithKline Beecham Biologicals vaccines.

	***Vitros* Anti-HBs assay Results**		
Reference Anti-HBs	**Reactive**	**Nonreactive**	**Total**
Reactive	183	0	183
Nonreactive	1	3	4
Total	184	3	187

Retrospective paired vaccination samples from twenty subjects who had received three injections of either the Engerix-B HBV vaccine (SmithKline-Beecham Biologicals) or the Recombivax HB vaccine (Merck & Co., Inc.) were evaluated at each of three study sites. Each sample pair consisted of matched pre- and approximately 30 days post-vaccination serum samples. A cut-off of ≥ 10 mIU/mL was used to define a reference anti-HBs reactive result. All three sites reported the same *Vitros* Anti-HBs assay results for all samples tested. The results are shown below for both the *Vitros* Anti-HBs assay and a quantitative anti-HBs reference method.

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Pre-Vaccination Panel

Vitros Anti-HBs Result	Reference Anti-HBs Result	
	-	Total
-	59	59
Total	59	59

	%(N)	95% Exact Confidence Interval
Negative Percent Agreement with the Reference Method	100 (59/59)	93.9 to 100

Post-Vaccination Panel

Vitros Anti-HBs Result	Reference Anti-HBs Result		
	+	-	Total
+	45	0	45
-	0	5	5
I	3	6	9
Total	48	11	59

	%(N)	95% Exact Confidence Interval
Positive Percent Agreement with the Reference Method	93.8 (45/48)	82.8 to 98.7
Negative Percent Agreement with the Reference Method	100 (59/59)	93.9 to 100

Combined Pre- and Post-Vaccination Panels

Vitros Anti-HBs Result	Reference Anti-HBs Result		
	+	-	Total
+	45	0	45
-	0	64	64
I	3	6	9
Total	48	70	118

	%(N)	95% Exact Confidence Interval
Positive Percent Agreement with the Reference Method	93.8 (45/48)	82.8 to 98.7
Negative Percent Agreement with the Reference Method	91.4 (64/70)	82.3 to 96.8

Based on all of the clinical laboratory study information presented, the following *Vitros* Anti-HBs assay result interpretation was established:

Vitros Anti-HBs assay Result	Status	Interpretation
< 0.50	Negative	Patient is presumed to be not immune to infection with HBV.
≥ 0.50 and < 1.20	Indeterminate	Unable to determine if anti-HBs is present at levels consistent with immunity. Patient's immune status should be further assessed by considering other clinical information.
≥ 1.20	Positive	Anti-HBs detected at > 10 mIU/mL. Patient is considered to be immune to infection with HBV.

10. Conclusions Drawn from Studies

The study data demonstrates that acceptable performance is obtained with the *Vitros* Anti-HBs assay when testing specimens collected in serum.

The *Vitros* Anti-HBs Reagent Pack and Calibrators can be stored up to 52 weeks at 2-8°C, while the Reagent Pack can be stored on-board the *Vitros* Analyzer (4-8°C, ≤40% relative humidity) for up to 8 weeks, and the Calibrators stored for up to 13 weeks at 2-8°C, or -20°C (with no more than one freeze-thaw cycle) after opening.

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The preservative systems that the *Vitros* Anti-HBs assay reagents uses are formulated with have been shown to meet USP 23 requirements at 45 weeks for the Assay Reagent, 31 weeks for the Conjugate Reagent and 27 weeks for Calibrator 1, which should be representative for Calibrators 2 and 3.

The *Vitros* Anti-HBs assay demonstrated precision estimates of <10% within day and between day for each site as well as across all sites, and <8% between replicate, <3% between day, <6% between site and <12% between lot when these variables were introduced.

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

Based on the results of the clinical laboratory studies, the *Vitros* Anti-HBs assay when used according to the provided directions should be safe and effective in the laboratory determination of susceptibility to HBV infection for individuals prior to or following HBV vaccination, or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis, in whom etiology is unknown.

11. CDRH Decision

Ortho-Clinical Diagnostics, Inc. has furnished information demonstrating that the *Vitros* Anti-HBs Reagent Pack and Calibrators, when used according to furnished directions, should be safe and effective for its indication for use.

FDA issued an approval order on September 29, 2000.

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

12. Approval Specifications

Directions for use: See Labeling

Warnings, Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, and Precautions.

Postapproval requirements and restrictions: See Approval Order.