Laboratory Procedure Manual

Analyte: Hepatitis B Surface Antibody (anti-HBs)

Matrix: Serum

Method: aHBs – Anti-HBs
VITROS Immunodiagnostic Products

Method No.:

First Published: February 24, 2011
Revised: *N/A*

As performed by: *Assay Development and Diagnostic Reference Laboratory (ADDRL)*
Laboratory Branch
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Important Information for Users

The National Center for HIV/AIDS, Hepatitis, STD and TB Prevention (NCHHSTP) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HEPB_S_F	LBXHBS	Hepatitis B surface antibody

Intended for Use in the United States

INSTRUCTIONS FOR USE aHBs

VITROS Immunodiagnostic Products

Anti-HBs Quantitative Reagent Pack

Anti-HBs

Version 2.1 Pub. No. GEM1208_EN

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

Intended Use

For the quantitative in vitro determination of total antibody to hepatitis B surface antigen (anti-HBs) in human serum using the VITROS ECi/ECiQ 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.

WARNING: ***Assay performance characteristics have not been established when the*** VITROS ***Anti-HBs Quantitative assay is used in conjunction with other manfacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.***

Summary and Explanation of the Assay

Viral hepatitis is a major public health problem of global importance with an estimated 300 million persistent carriers of HBV worldwide.[1] Infection with HBV results in a wide spectrum of acute and chronic liver diseases that may lead to cirrhosis and hepatocellular carcinoma.[2]
HBV infection produces an array of unique antigens and antibody responses that, in general follow distinct serological patterns. Hepatitis B surface antigen (HBsAg), derived from the viral envelope, is the first antigen to appear following infection. The development of neutralizing anti-HBs occurs in 90% of patients infected with HBV and is associated with resolution of the infection and protective immunity.[3]
Individuals who have resolved their HBV infection usually demonstrate both anti-HBs and antibody to hepatitis B core antigen (anti-HBc) in their serum. The absence of both anti-HBs and anti-HBc is indicative of susceptibility to HBV infection, and can identify individuals who may benefit from vaccination.[4]
Both plasma derived and recombinant protein based vaccines have been developed and shown to be effective in inducing immunity to HBV through production of antibodies to HBsAg. Anti-HBs testing is useful for identifying HBV susceptible individuals in pre- and post-vaccination screening programs.[5, 6]
A variety of standard immunological techniques have been used for the detection of anti-HBs including immuno-diffusion,[7] "sandwich" immuno-radiometry,[8] electroimmuno-osmophoresis,[9] and passive agglutination,[10] or agglutination inhibition.[11] The more recent solid phase "sandwich" enzyme-labeled immunoassays provide a rapid, specific, and highly sensitive test system for the measurement of anti-HBs.

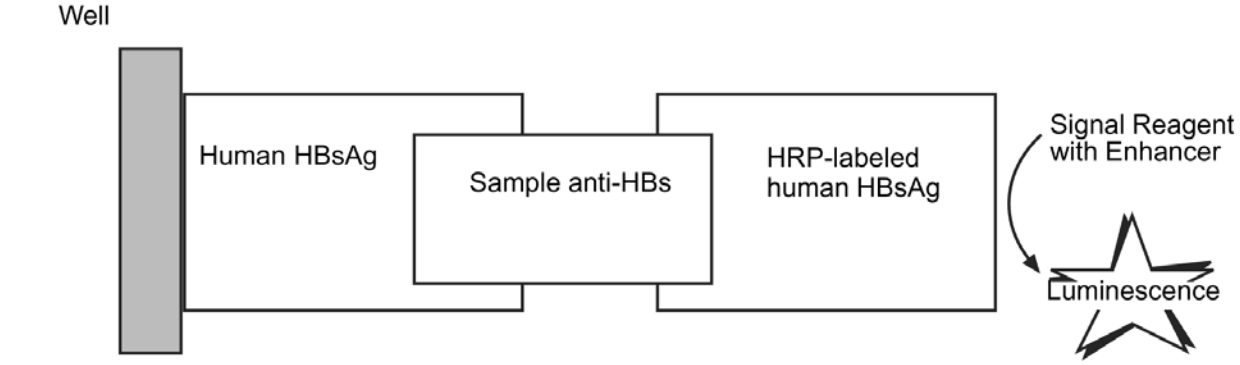
Principles of the Procedure

The VITROS Anti-HBs Quantitative assay is performed using the VITROS Anti-HBs Quantitative Reagent Pack and VITROS Immunodiagnostic Products Anti-HBs Calibrators on the VITROS ECi/ECiQ Immunodiagnostic System.
An immunometric technique is used. This involves the reaction of anti-HBs in the sample with HBsAg (ad and ay subtypes) coated onto the wells. A horseradish peroxidase (HRP)-labeled HBsAg conjugate (ad and ay subtypes) then complexes with the bound anti-HBs forming an "antigen sandwich." Unbound materials are removed by washing.
A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells.[13] The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent increases the level and duration of the light produced. The light signals are read by the VITROS ECi/ECiQ

Immunodiagnostic System. The amount of HRP conjugate bound is directly proportional to the concentration of anti-HBs present.

Assay Type	Assay Time and Temperature	
Immunometric assay	Incubation time:	45 minutes
	Time to first result:	55 minutes
	Temperature:	37°C

Reaction Scheme



Warnings and Precautions

For in vitro diagnostic use only.

WARNING: Potentially Infectious Material

The conjugate reagent and coated wells provided as part of the* VITROS *Anti-HBs Quantitative Reagent Pack contain purified native hepatitis B surface antigen (HBsAg) obtained from donors who were tested individually and found to be negative for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays). The purified HBsAg has been heat inactivated (10 hours at 60°C). Treat as if capable of transmitting infection. Human blood products provided as components of this pack, and of the* VITROS *Anti-HBs Calibrators, have been obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen (HBsAg), and for antibodies to HIV 1+2 and hepatitis C virus (HCV) using FDA approved methods (enzyme immunoassay). Treat as if capable of transmitting infection.
Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handling of samples and assay components, their use, storage and solid and liquid waste disposals should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate national biohazard safety guideline[14] or regulation (e.g. NCCLS guideline M29. [15]).

WARNING: Contains Kathon

The* VITROS *Anti-HBs conjugate reagent and assay reagent contain Kathon. R43 May cause sensitization by skin contact. R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S24 Avoid contact with skin. S37 Wear suitable gloves.

Reagents

Reagent Pack Contents

One VITROS Anti-HBs Quantitative Reagent Pack; 100 tests (CAT No. 680 1925) contains:
· 100 human HBsAg (ad and ay subtypes) coated wells
· 13.3 mL conjugate reagent: human HBsAg (ad and ay subtypes)-HRP conjugate in phosphate buffered saline with human plasma, protein stabilizers and antimicrobial agent (Kathon, 2% w/v)
· 6.2 mL assay reagent: EDTA phosphate buffered saline with antimicrobial agent (Kathon, 1%, w/v)

Reagent Pack Handling

· The reagent pack is supplied ready for use.
· The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading on the VITROS ECi/ECiQ Immunodiagnostic System.
· Avoid agitation, which may cause foaming or the formation of bubbles.
· Do not use visibly damaged product.

Reagent Pack Stability

When stored and handled as specified in the package labeling, the VITROS Anti-HBs Quantitative Reagent Pack is suitable for use until the expiration date printed on the outside of the carton.

Reagent Pack Storage and Preparation

· Store the unopened reagent pack refrigerated at 2°–8°C (36°–46°F). Do not freeze.
· Load reagent packs directly from refrigerated storage to minimize condensation.
· Use opened reagent packs within 8 weeks.
· Store opened reagent packs in the VITROS ECi/ECiQ Immunodiagnostic System reagent supply, or refrigerated at 2°–8°C (36°–46°F) in a sealed reagent pack storage box that contains dry desiccant.

Specimen Collection and Preparation

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum

Specimens Not Recommended

· Turbidity in samples may affect assay results.
· Do not use plasma samples.

Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies.[16] Because of the variety of sample collection devices available it is not possible to issue a definitive statement on the performance of VITROS Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer's instructions and is compatible with this assay.

Specimen Collection and Preparation

· Collect specimens using standard procedures.[17]
· The VITROS Anti-HBs assay uses 80 µL of sample for each determination.
· For details on minimum fill volume of sample cups or containers, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.
· Mix samples, calibrators, and controls by inversion and bring to 15° –30° C (59° –86° F) before use.
· Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result.

Handling and Storage Conditions

· Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.
· The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for further information. Return to 2° –8°C (36° –46°F) as soon as possible after use or load sufficient volume for a single determination.
· The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing serum specimens:[18]

– Store samples at 22°C (72°F) for no longer than 8 hours.
– If the assay will not be completed within 8 hours, refrigerate the serum at 2° –8°C (36° –46°F).
– If the assay will not be completed within 48 hours, or for shipment, freeze the serum at or below -20°C (-4°F).
· Samples are not to be repeatedly frozen and thawed because this can cause analyte deterioration. Samples are to be thawed only once.

Assay Procedure

Materials Required But Not Provided

The following items are required to perform the VITROS Anti-HBs assay:
· VITROS ECi/ECiQ Immunodiagnostic System
· VITROS Anti-HBs Calibrators
· VITROS Immunodiagnostic Products Signal Reagent
· VITROS Immunodiagnostic Products Universal Wash Reagent
· Quality control materials, such as VITROS Immunodiagnostic Products Anti-HBs Controls
· VITROS Immunodiagnostic Products High Sample Diluent B Reagent Pack
· VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

Operating Instructions

Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for complete instructions on the operation of your VITROS System.

Sample Dilution

Serum samples with concentrations greater than the reportable range may be automatically diluted up to 400 fold (1 part sample with 399 parts diluent) by the VITROS ECi/ECiQ Immunodiagnostic System with VITROS Immunodiagnostic Products High Sample Diluent B Reagent Pack prior to assay. Refer to the High Sample Diluent B Reagent Pack instructions for use.

Calibration

Required Calibrators

VITROS Anti-HBs Calibrators

Traceability

The calibration range of the VITROS Anti-HBs Quantitative assay is 0 – 1000 mIU/mL. Calibration of the VITROS Anti-HBs Quantitative assay is traceable to the World Health Organization (WHO) First International Reference Preparation for Antibody to HBsAg (1977).

Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of VITROS Anti-HBs Calibrators.

Calibration Procedure

· Calibration must be performed using calibrators of the same lot number as the reagent pack.
· Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for detailed instructions on the calibration process.

When to Calibrate

· Calibrate when the lot of reagent pack and calibrator changes.
· Calibrate every 28 days.
· The calibration report should be used in conjunction with the quality control results to determine the validity of the calibration. Quality control results must be within the stated range.

The VITROS Anti-HBs Quantitative assay may also need to be recalibrated:
· After specified service procedures have been performed (see the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide).
· If quality control results are consistently outside of the manufacturer's or your acceptable range.

For additional information on calibration management, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.

Calibration Model

Modified four-parameter logistic curve fit.
Results are automatically calculated by the VITROS ECi/ECiQ Immunodiagnostic System.

Reportable Range (Dynamic Range)

Using the VITROS Anti-HBs Quantitative assay, the VITROS ECi/ECiQ Immunodiagnostic System will report values of 0 – 1000 mIU/mL. Values between 0 mIU/mL and the Limit of Detection (LoD) should be interpreted as not having detectable anti-HBs.

Assay Limit of Detection

The lowest amount of anti-HBs that can be detected with the VITROS Anti-HBS Quantitative assay was determined in accordance with NCCLS EP17.[19] Based upon 274 positive determinations, the Limit of Detection (LoD) is 4.23 mIU/mL of anti-HBs, with a 95% probability of obtaining a measurable response at that level. A Limit of Blank (LoB) of 3.08 mIU/mL was used.

Quality Control

Procedure Recommendations

· Choose control levels that check the clinically relevant concentrations. The recommendation is to run a negative control and a positive control close to the anti-HBs decision point (10 mIU/mL)
· To verify system performance, analyze control materials:
– After calibration
– At least once every 24 hours
– After specified service procedures or maintenance to critical parts or subsystems that might influence the performance of the assay (see the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide).
· Analyze quality control materials in the same manner as patient specimens.
· If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within the acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
· For more detailed information on quality control procedures, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.
· Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control.[20]
· Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

Quality Control Material Selection

Choose control material that has the composition of the patient sample matrix being analyzed.[20]
VITROS Anti-HBs Controls are recommended for use with the VITROS ECi/ECiQ Immunodiagnostic System. The performance of commercial control fluids should be evaluated for compatibility with this assay before they are used for quality control. Appropriate quality control value ranges should be established for all commercially available quality control materials used with the VITROS Anti-HBs Quantitative assay.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Interpretation of Results and Expected Results

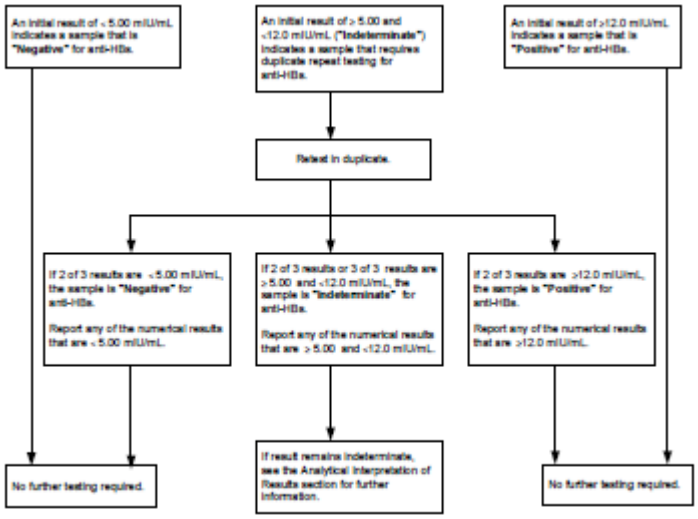
Analytical Interpretation of Results

· A result of < 4.23 mIU/mL indicates that the result is below the assay's Limit of Detection (LoD). The sample is "Negative" for anti-HBs and the individual is not immune to HBV infection.
· A result of ≥4.23 mIU/mL and < 5.00 mIU/mL indicates with 95% confidence that a sample contains anti-HBs, but not at levels consistent with protective immunity against HBV infection.

· A result of ≥12.0 mIU/mL indicates that a sample is “Positive” for anti-HBs. This result is consistent with levels of anti-HBs at >10 mIU/mL, which indicates that anti-HBs has been detected at levels consistent with protective immunity against HBV infection. [4, 21,22]
· A specimen with a result of ≥ 5.00 mIU/mL and <12.0 mIU/mL indicates that a sample is “Indeterminate” for anti-HBs and should be retested in duplicate. If both repeats are < 5.00 mIU/mL, the specimen is negative for anti-HBs. If both repeats are ≥12.0 mIU/mL , the specimen is positive for anti-HBs. The result is indeterminate if one or both replicate results are ≥ 5.00 mIU/mL and < 12.0 mIU/mL. If a result remains indeterminate, the immune status of the individual should be further assessed by considering other factors, such as clinical status, follow-up testing, associated risk factors, and the use of additional diagnostic information.
· Results obtained with the VITROS Anti-HBs Quantitative assay may not be used interchangeably with values obtained with different manufacturers’ assay methods.

Testing Algorithm

Final results should be manually interpreted using the algorithm below.



The clinical significance of quantitative values reported ≥12.0 mIU/mL has not been determined, other than the individual is presumed to be immune to HBV infection. The clinical significance of quantitative values reported ≥4.23 mIU/mL and <5.00 mIU/mL has not been determined, other than the individual is considered to be not immune to HBV infection.

VITROS Anti-HBs assay Result	Result Text	Clinical Interpretation of Immune Status
<5.00 mIU/mL	Negative	Patient is considered to be not immune to infection with HBV.
≥ 5.00 mIU/mL and <12.0 mIU/mL	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 or retesting another specimen drawn at a later time.
≥12.0 mIU/mL	Positive	Anti-HBs detected at >10 mIU/mL. Patient is considered to be immune to infection with HBV. It has not been determined what the clinical significance is for values greater than ≥ 12 mIU/mL, other than the individual is considered to be immune to HBV infection.

Expected Results

Approximately 61% (1078/1775) of the prospective subjects participating in the VITROS Anti-HBs Quantitative assay 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 five to 96 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS Anti-HBs Quantitative

assay was positive in 27% of the individuals in this group. The percent VITROS Anti-HBs positive 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 positive, negative, 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 was not reported for three subjects.

Limitations of the Procedure

· Assay performance characteristics have not been established when the VITROS Anti-HBs Quantitative assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
· Assay performance characteristics have not been established for the use of the VITROS Anti-HBs Quantitative assay as an aid in determining susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.
· The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture.
· This assay does not differentiate between a vaccine induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
· Individuals that have received blood component therapy, e.g., whole blood, plasma, immune globulin administration, during the previous 3 to 6 months may have a false reactive anti-HBs result due to passive transfer of anti-HBs. [4]
· Results from immunosuppressed individuals should be interpreted with caution.
· Individuals possessing IgM anti-rubella virus may have falsely high results with the VITROS Anti-HBs Quantitative assay.
· Assay performance characteristics have not been established for any other specimen matrix than serum.
· Turbidity may affect assay results.
· The prevalence of the analyte will affect the assay's predictive value.

Performance Characteristics

Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS Anti-HBs Quantitative 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 five to 96 years. The HBV disease classification for each subject was determined by a 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). 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 Quantitative assay was assessed relative to the reference anti-HBs result and the specimen classification using serum samples from a total of 1761 subjects.

Results by Specimen Classification

The data were analyzed following the assignment of specimen classification based upon the positive (+)/negative (-) patterns for the six HBV reference markers. The table below summarizes how these classifications were derived. There were 22 unique reference marker patterns observed in the VITROS Anti-HBs clinical study.

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

Comparison of Results

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. In the clinical study, specimens that had VITROS Anti-HBs indeterminate results ≥8.00 mIU/mL and <12.0 mIU/mL were retested in duplicate. Specimens with indeterminate results ≥5.00 mIU/mL and <8.00 mIU/mL were not retested.

	Anti-HBs Reference Result								
	-				+				
	VITROS Anti-HBs Result				VITROS Anti-HBs Result				
HBV Classification	-	+	I*	I**	-	+	I*	I**	Total
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	8	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.

Percent Agreement

The table below summarizes the percent agreement between the VITROS Anti-HBs Quantitative assay and the anti-HBs reference assay for each specimen classification, and provides the upper and lower 95% exact confidence bounds.

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Overall	93.21 (494/530)	90.72–95.20	94.39 (1162/1231)	92.96–95.61
Acute	NA	NA	100.0 (21/21)	83.89–100.0
Chronic	33.33 (1/3)	0.84–90.57	95.12 (39/41)	83.47–99.40
Early Recovery	88.89 (8/9)	51.75–99.72	66.67 (32/48)	51.59–79.60
Recovery	95.17 (138/145)	90.31–98.04	NA	NA
Recovered	88.74 (134/151)	82.59–93.30	84.34 (70/83)	74.71–91.39
Uninterpretable	100.0 (3/3)	29.24–100.0	87.50 (7/8)	47.35–99.68
HBV Vaccine Response	95.89 (210/219)	92.34–98.10	NA	NA
Not Previously Infected	NA	NA	96.41 (993/1030)	95.08–97.46

Clinical Performance with Individuals Who Have Received Hepatitis B Vaccine

A retrospective study was conducted to evaluate a total of 187 serum samples from subjects who had received a full course of injections (three) of either *SmithKline-Beecham Biologicals Engerix-B* □ HBV vaccine or *Merck & Co., Inc. Recombivax HB*

vaccine. VITROS Anti-HBs Quantitative assay positive results were obtained for 184 samples (98.4%, 184/187). This was similar to the rate of positive results observed with a quantitative anti-HBs reference method (97.9%, 183/187). No statistically significant difference was noted with the VITROS Anti-HBs results for either vaccine.

VITROS Anti-HBs Result	Reference Anti-HBs Result		
	+	-	Total
+	183	1	184
-	0	3	3
Total	183	4	187

	% (N)	95% Exact Confidence Interval
Positive Percent Agreement with the Reference Method	100.0 (183/183)	98.00–100.0%
Negative Percent Agreement with the Reference Method	75.00 (3/4)	19.41–99.37%

Clinical Performance with Matched Pre- and Post-Vaccination Samples

In another study, pre- and post-vaccination samples from twenty individuals who had received recombinant HBV vaccine were tested with the VITROS Anti-HBs Quantitative assay at three external sites. All three sites reported the same VITROS Anti-HBs Quantitative assay results for all samples tested. The results are shown below for both the VITROS Anti-HBs Quantitative assay and a quantitative anti-HBs reference method.

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	100.0 (59/59)	93.94–100.0%

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	93.75 (45/48)	82.80–98.69%
Negative Percent Agreement with the Reference Method	45.45 (5/11)	16.75–76.62%

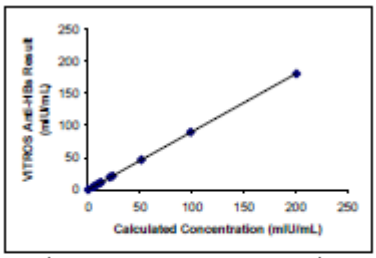
Combined Pre- and Post-Vaccination Panels

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
Overall Positive Percent Agreement with the Reference Method	93.75 (45/48)	82.80–98.69%
Overall Negative Percent Agreement with the Reference Method	91.43 (64/70)	82.27–96.79%

Analytical Sensitivity

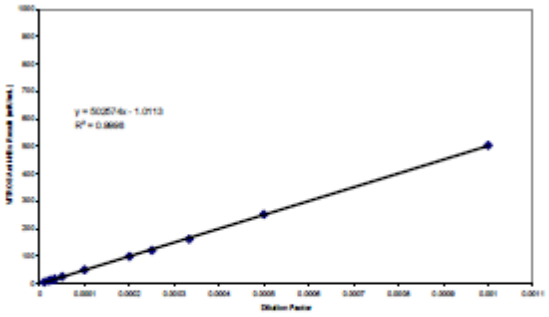
VITROS Anti-HBs Quantitative assay results were measured for dilutions of the WHO First International Reference Preparation for Antibody to HBsAg (1977) using 18 determinations and 3 reagent batches. A linear regression of the mean VITROS Anti-HBs result versus the calculated concentration of each WHO Reference Preparation dilution was used to determine the anti-HBs concentration (10.97 mIU/mL) at the VITROS Anti-HBs Quantitative assay value of 10.0 mIU/mL.



rSquare	1.0000
Intercept	0.101
Slope	0.902
Calculated value at cut-off	10.97 mIU/mL

Dilution Linearity

The linearity of diluting patient samples was assessed by diluting a pool of high titer hepatitis B immunoglobulin created from 100 to 500 patient samples into negative plasma and testing each dilution in duplicate. A linear regression of the VITROS Anti-HBs Quantitative assay results at each dilution was used to show that dilution of antibody present in patient samples is linear.



rSquare	0.9998
Intercept	-1.0113
Slope	502574

Potentially Cross-Reacting Subgroups

The specificity of the VITROS Anti-HBs Quantitative assay was evaluated by testing 209 samples from 16 potentially crossreacting sub-groups. All of the samples were previously classified as anti-HBs negative in other commercially available assays. Samples found to be ≥10.0 mIU/mL by the VITROS Anti-HBs Quantitative assay were retested in duplicate. A summary of the results is given in the table below.

Clinical Category	Number Samples Tested	VITROS Anti-HBs assay Result < 5.00 mIU/mL	VITROS Anti-HBs assay Result ≥ 5.00 mIU/mL and < 12.0 mIU/mL	VITROS Anti-HBs assay result ≥12.0 mIU/mL
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 ≥ 12.0 mIU/mL.
*** Sample coagulated - retest not possible

Substances that do not Interfere

As recommended by NCCLS Protocol EP7, [23] the VITROS Anti-HBs assay was evaluated for interference by testing the following substances. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with anti-HBs to a concentration near 10 mIU/mL. None of the compounds at the levels tested were found to interfere with the clinical interpretation of the assay.

Compound	Compound Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Hemoglobin	0.31 mmol/L	500 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

Precision

Precision of the VITROS Anti-HBs quantitative assay was evaluated on the VITROS ECi/ECiQ Immunodiagnostic System, using one reagent pack and calibrator kit lot. At least two replicates each of a seven-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.

Test Site	Mean VITROS Anti-HBs assay result (mIU/mL)	Within Day*		Between Day**		Total***		No. Observ.	No. of Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Site 1	10.1	0.136	1.4	0.388	3.9	0.411	4.1	40	20
	24.9	0.321	1.3	0.731	2.9	0.798	3.2	40	20
	83.4	0.778	0.9	2.029	2.4	2.172	2.6	40	20
	337	2.246	0.7	6.047	1.8	6.451	1.9	40	20
	484	4.892	1.0	6.455	1.3	8.099	1.7	40	20
	752	30.666	4.1	15.420	2.1	34.325	4.6	40	20
	933	23.035	2.5	10.436	1.1	25.289	2.7	40	20
Site 2	10.2	0.169	1.7	0.284	2.8	0.330	3.2	40	20
	25.2	0.335	1.3	0.476	1.9	0.582	2.3	40	20
	84.6	0.861	1.0	0.563	0.7	1.029	1.2	40	20
	342	4.260	1.2	2.975	0.9	5.196	1.5	40	20
	488	5.945	1.2	5.487	1.1	8.090	1.7	40	20
	782	12.503	1.6	4.802	0.6	13.393	1.8	40	20
	944	10.789	1.1	10.999	1.2	15.407	1.6	40	20
Site 3	10.1	0.114	1.1	0.544	5.4	0.555	5.5	40	20
	25.1	0.478	1.9	0.473	1.9	0.672	2.7	40	20
	84.5	0.707	0.8	1.103	1.3	1.310	1.6	40	20
	343	5.191	1.5	2.116	0.6	5.606	1.6	40	20
	490	5.601	1.1	5.958	1.2	8.177	1.7	40	20
	781	8.990	1.2	14.263	1.9	16.860	2.2	40	20
	940	14.129	1.5	12.933	1.4	19.154	2.0	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 day

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 mIU/mL	Between Site*		Between Lot**		Total***		No. Observ.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
8.20	0.36	5.8	0.00	0.0	0.75	12.0	274
9.50	0.36	3.8	1.07	11.3	1.27	13.4	271
10.5	0.48	4.6	0.15	1.5	0.85	8.1	270
41.7	1.66	4.0	4.99	12.0	5.50	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

The data presented in both studies are a representation of assay performance based on the studies described. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect the reproducibility of assay results.

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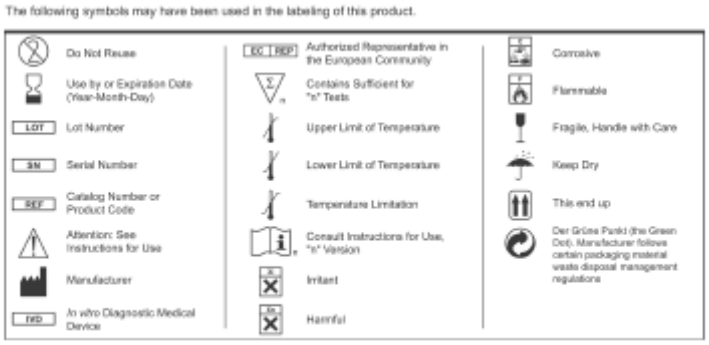
Glossary of Symbols



Revision History

Date of Revision	Version	Description of Technical Changes*
2007-12-18	2.1	Updated Sample Dilution section to include full name of High Sample Diluent B Reagent Pack Registered address updated to 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP12 4DP, United Kingdom Address Block: CHIRON Corporation changed to Novartis Vaccines and Diagnostics, Inc.
2005-02-02	2.0	Interpretation of Results: • Testing Algorithm: Added boxes and text for clarification when reporting numeric results Performance Characteristics: • Analytical Sensitivity: Revised text to "results were measured for dilutions of" from "results around the cut-off were compared to dilutions of" Revised text-to "value" from "cut-off" of 10 mIU/mL Glossary of Symbols: Added new table. Table updated for the addition of "n" to the following symbols: • Contains Sufficient for "n" Tests • Consult Instructions for Use, "n" version
2004-09-09	1.0	Initial version of Instructions for Use

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature _____ Obsolete Date _____

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