Vitros Immunodiagnostic Products Anti-HBs Calibrators

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

Intended Use

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 WHO First International Reference Preparation for Antibody to HBsAg (1977).

Principles of Procedure

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 which 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[1] values or control ranges to determine the validity of the calibration. Recalibration is required after a pre-determined calibration interval (refer to the *Vitros* Anti-HBs Reagent Pack calibration instructions) or when a different reagent lot is loaded.

Warnings and Precautions

For *In Vitro* Diagnostic Use Only

Warning - Potentially Infectious Material
Human blood products provided as components of this pack have been obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays).

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 disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation. [2,3]

Materials Provided

- 1 set of Anti-HBs Calibrators 1, 2 and 3 (2 mL, recalcified human plasma with antimicrobial agent, Bronidox 1.0%, nominal results of 0, 3, 25, values encoded on the lot calibration card).
- Lot calibration card.
- Protocol card.
- 24 calibrator bar code labels (8 for each calibrator)

Reagent Preparation and Storage

Anti-HBs Calibrators are supplied ready for use. Store unopened at 2-8 °C (36°–46°F). Do not use beyond the expiration date. After opening, store for up to 13 weeks at 2-8 °C (36°–46°F) or 13 weeks at -20 °C (-4 °C) (with no more than 1 freeze-thaw cycle).

Quality Control and Procedural Notes

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15-30 °C (59°–86°F) before use. Each pack contains sufficient volume for a minimum of 6 determinations of each calibrator.

- Evaporation will occur when calibrators are stored open on board the *Vitros* System, refer to the Operators Guide, Chapter 6, Preparing Samples. Return to 2-8 °C (36°–46°F) as soon as possible after use, or load only sufficient volume for a single determination. Calibrators may be aliquoted into appropriate alternative stoppered containers, which may be bar coded with the labels provided

Procedure

For further information refer to the *Vitros* Anti-HBs Reagent Pack instructions for use. For detailed instructions on calibration refer to the *Vitros* Immunodiagnostic System Operator's Guide, Chapter 5, Performing Calibration.

References

1. NCCLS. *Specification for Immunological Testing for Infectious Diseases; Approved Guideline.* NCCLS Document I/LA 18-A (ISBN1-56238-251-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylavania 19087, 1994.

2. CDC-NIH. *Biosafety in Microbiological and Biomedical Laboratories – 3rd Edition.* HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C., 1993.

3. NCCLS. *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline.* NCCLS Document M29-A (ISBN 1-56238-339-6). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1997.

VITROS Immunodiagnostic Products

METHODOLOGY SHEET

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

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

Intended Use

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, ~~but~~ in whom etiology is unknown.

WARNING:

- ***This assay has not been FDA cleared or approved for the screening of blood or plasma donors.***
- ***Assay performance characteristics have not been established when the VITROS Anti-HBs 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.[12]

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Principles of the Procedure

The *Vitros* Anti-HBs assay is performed using the *Vitros* Anti-HBs Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HBs Calibrators on the *Vitros* ECi 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 System. The amount of HRP conjugate bound is indicative of the concentration of anti-HBs present in the sample.

Assay Type

Immunometric assay

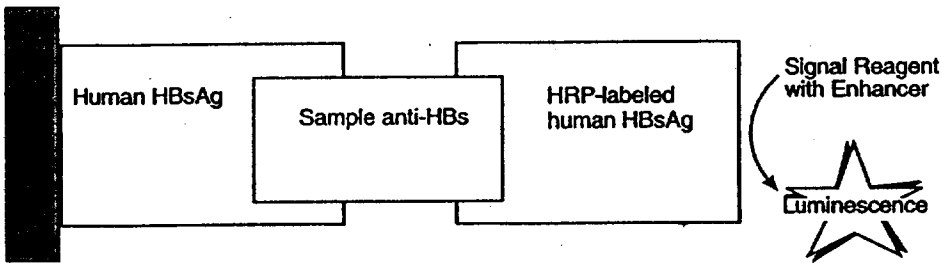
Assay Time and Temperature

Incubation time: 45 minutes

Time to first result: 55 minutes

Temperature: 37°C

Reaction Scheme



Warnings and Precautions

For in vitro diagnostic use only.

The conjugate reagent and coated wells provided as part of this 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, EIA). 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 HBsAg, and for antibodies to HIV 1+2 and HCV (using FDA approved EIA).

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 disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation.[14,15]

WARNING: ***The VITROS Anti-HBs conjugate reagent contains Kathon (2% w/v). The total Kathon content in the Reagent Pack is 460 mg. Kathon may cause sensitization by skin contact. Avoid contact with skin.***

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Reagents

Reagent Pack Contents

One *Vitros* Anti-HBs Reagent Pack; 100 tests (CAT No. 680 1319) contains:

- 100 human HBsAg (ad and ay subtypes) coated wells
- 18.5 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)
- 9.0 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.
- Reagent packs do not need mixing.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Pack Stability

When stored and handled as specified in the package labeling, the *Vitros* Anti-HBs 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 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 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.

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Specimen Collection and Preparation (continued)

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 System Operator's Guide for further information.
- 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).
- Serum 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 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 Reagent Pack Storage Box (optional) with desiccant

Operating Instructions

Refer to the *Vitros* ECi System Operator's Guide for complete instructions on the operation of your *Vitros* ECi System.

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Calibration

Required Calibrators

Vitros Anti-HBs Calibrators

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 System Operator's Guide for detailed instructions on how to calibrate.

When to Calibrate

- Calibrate when the lot of reagent pack and calibrator changes
- Calibrate every 28 days

The *Vitros* Anti-HBs assay may also need to be recalibrated:

- After specified service procedures have been performed (see the *Vitros* ECi System Operator's Guide)
- If quality control results are consistently outside of the manufacturer's or your acceptable range.

For additional information on when to calibrate, refer to the *Vitros* ECi System Operator's Guide.

Traceability

Calibration of the *Vitros* Anti-HBs assay is traceable to the WHO First International Reference Preparation for Antibody to HBsAg (1977).

Calibration Model

Modified four-parameter logistic curve fit.

Quality Control

Procedure Recommendations

- Choose control levels that check performance at clinically relevant points. 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 performance of the assay (see the *Vitros* ECi 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 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 System Operator's Guide.
- Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control recommendations.[19]
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Quality Control Material Selection

Vitros Anti-HBs Controls are recommended for use with the *Vitros* ECi System. The performance of other 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 assay.

Quality Control Material Preparation and Storage

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

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Interpretation of Results and Expected Results

Interpretation of Results

- Calibration of the *Vitros* Anti-HBs assay is traceable to the WHO First International Reference Preparation for Antibody to HBsAg (1977). A value of 10mIU/mL was normalized to a *Vitros* Anti-HBs assay result of 1.00. The accepted criterion for immunity to HBV is ≥ 10 mIU/mL of anti-HBs, with mIU defined by the WHO Reference Preparation.[20]
- A result of < 0.50 indicates that a sample is "Negative" for anti-HBs. A negative result indicates that anti-HBs has not been detected at levels consistent with immunity.
- A result of ≥ 1.20 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]
- A specimen with a result of ≥ 0.50 and < 1.20 indicates that a sample is "Indeterminate" for anti-HBs and should be retested in duplicate. If both repeats are < 0.50, the specimen is negative for anti-HBs. If both repeats are ≥1.20, the specimen is positive for anti-HBs. The result is indeterminate if one or both replicate results are ≥ 0.50 and < 1.20. 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 assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- The magnitude of a *Vitros* Anti-HBs assay result cannot be correlated to an endpoint titer.

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.

Interpretation of Results and Expected Results (continued)

Expected 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 five 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 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.

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Limitations of the Procedure

- This device is not intended for use in screening blood bank donors.
- Assay performance characteristics have not been established when the *Vitros* Anti-HBs 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 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 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.

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Performance Characteristics

Clinical Performance

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 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 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						
HBsAg	HBeAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBe	Anti-HBs (≥10 mIU/mL)	HBV Classification
+	+	+	+	+	-	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

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Performance Characteristics (continued)

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 ≥ 0.80 and < 1.20 were retested in duplicate. Specimens with indeterminate results ≥ 0.50 and < 0.80 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	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.

Percent Agreement

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.

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

Performance Characteristics (continued)

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 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 assay at three external sites. 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.

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%

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Performance Characteristics (continued)

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. All of the samples were previously classified as anti-HBs negative in other commercially available assays. Samples found to be ≥ 1.00 by the *Vitros* Anti-HBs 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 < 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 from the same patient and were initially ≥ 1.20.
† Sample coagulated - retest not possible

Substances that do not Interfere

As recommended by NCCLS Protocol EP7,[22] 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

Performance Characteristics (continued)

Precision

Precision was evaluated on a different *Vitros* ECi System at three external sites, using one lot of reagent. At least two replicates 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.

	Mean *Vitros* Anti-HBs 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

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

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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