Laboratory Procedure Manual

Analyte: Hepatitis B Core Antibody

Matrix: Serum

Method: aHBc – Anti-HBc
VITROS Immunodiagnostic Products (REF 680 1428)

Method No.:

First Published: February 24, 2011
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As performed by: *Assay Development and Diagnostic Reference Laboratory (ADDRL)*
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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.

February 24, 2011

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
HEPBD_F	LBXHBC	Hepatitis C core antibody

Intended for Use in the United States

INSTRUCTIONS FOR USE aHBc
VITROS Immunodiagnostic Products
Anti-HBc Reagent Pack

REF 680 1428

Version 3.0 Pub. No. J20866

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

Intended Use

For the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis B core antigen (total anti-HBc) in human adult and pediatric serum and plasma (EDTA and citrate) and neonate serum using the VITROS ECi/ECiQ Immunodiagnostic System.
Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection. The presence of anti-HBc may be used as an aid in the determination of exposure to HBV infection for individuals prior to HBV vaccination.

WARNING: ***This assay has not been FDA cleared or approved for the screening of blood or plasma donors.***

Summary and Explanation of the Assay

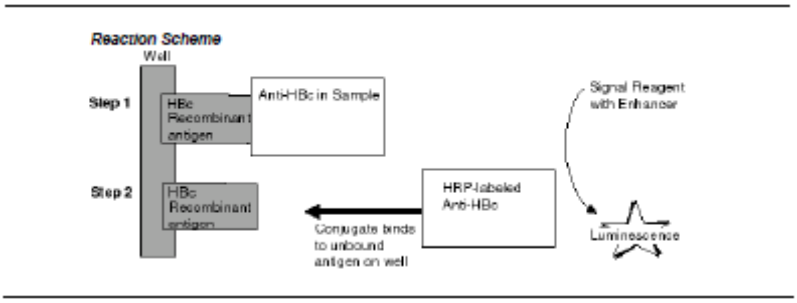
The VITROS Anti-HBc assay can be used to detect antibodies against hepatitis B core antigen (anti-HBc) in serum and plasma following exposure to infectious hepatitis B virus (HBV)[1, 2]. Anti-HBc is detectable shortly after the appearance of hepatitis B surface antigen (HBsAg). As the appearance of anti-HBsAg may be delayed after HBsAg clearance, anti-HBc is sometimes the only serological marker for HBV infection and potentially infectious blood[3]. Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection.

Principles of the Procedure

The VITROS Anti-HBc assay is performed using the VITROS Anti-HBc Reagent Pack and VITROS Immunodiagnostic Products Anti-HBc Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System.
A competitive immunoassay technique is used. This involves the reaction of anti-HBc in the sample with hepatitis B core antigen (HBcAg) coated wells. Unbound sample is removed by washing. Horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-HBc) is then allowed to react with the remaining exposed HBcAg on the well surface. Unbound conjugate is removed by washing.
The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells[4]. 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 indicative of the concentration of anti-HBc present in the sample.

Assay Type Assay Time and Temperature

Assay Type	Assay Time and Temperature	
Competitive	Incubation time:	46 minutes
	Time to first result:	55 minutes
	Temperature:	37 °C



Warnings and Precautions

For *in vitro* diagnostic use only.

Warning: ***Potentially Infectious Material***

· Treat as if capable of transmitting infection.
· Handling of samples and assay components, their use, storage and solid and liquid waste disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation. (e.g. CLSI Guideline M29 [5,6]).

Human blood products provided as components of this pack, and of the VITROS Anti-HBc Calibrator, 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, 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.

WARNING:[7] ***Contains Kathon:***

The assay reagent in the VITROS ***Anti-HBc Reagent Pack contains Kathon (0.5% w/w).***

The conjugate reagent in the VITROS ***Anti-HBc Reagent Pack contains Kathon (0.5% w/w).***
R36/38 – Irritating to eyes and skin.
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.
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37/39 – Wear suitable gloves and eye/face protection.

Reagents

Reagent Pack Contents

One VITROS Anti-HBc Reagent Pack, 100 tests (CAT No. 680 1428) contains:
· 100 coated wells [recombinant HBcAg derived from bacteria (*E.coli*) coated at 1.5 ng per well].
· 14.6 mL assay reagent [buffer with newborn calf serum, bovine gamma globulins and anti-microbial agent (0.5% Kathon w/w)].
· 20.6 mL conjugate reagent (HRP-mouse monoclonal anti-HBc, 0.1 μ g/mL, in buffer with mouse serum, human plasma and anti-microbial agent (0.5% Kathon w/w).

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-HBc 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, EDTA or citrated plasma.

Specimens Not Recommended

Turbidity in samples may affect results.

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[8]. 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[9].
- The VITROS Anti-HBc assay uses 50 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, calibrator, 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 an erroneous result.
- Do not use heat inactivated samples.

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.

- The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing specimens:[10]

  - Store samples at 22 °C (72 °F) for no longer than 8 hours.
  - If the assay will not be completed within 8 hours, refrigerate samples at 2–8 °C (36–46 °F).
  - If the assay will not be completed within 48 hours, or for shipment, freeze samples 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-HBc assay:
· VITROS ECi/ECiQ Immunodiagnostic System
· VITROS Anti-HBc Calibrator
· VITROS Immunodiagnostic Products Signal Reagent
· VITROS Immunodiagnostic Products Universal Wash Reagent
· Quality control materials, such as VITROS Immunodiagnostic Products Anti-HBc Controls
· VITROS Immunodiagnostic Products High Sample Diluent B
· 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 ECi /ECiQ Immunodiagnostic System.

Sample Dilution

Rare patient samples occur that give high result ratios (s/c) greater than the normal negative population and which may be negative or positive for anti-HBc total antibody. These samples are defined by a result of greater than or equal to 4.8 s/c ratio and are most likely attributed to an unidentified interferent. For samples with results flagged "Equivocal", dilute a measured aliquot with an appropriate volume of VITROS High Sample Diluent B to give a 1 in 20 dilution. For example, dilute 100 µL sample with 1.90 mL VITROS High Sample Diluent B and vortex mix. The VITROS Immunodiagnostic System **does not need to be programmed** for the dilution factor when testing the diluted sample using the VITROS Anti-HBc Reagent Pack. The results for a diluted sample should be interpreted as described in the VITROS Anti-HBc Reagent Pack Instructions for Use.

Calibration

Required Calibrator

VITROS Anti-HBc Calibrator

Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of the VITROS Anti-HBc Calibrator.

Calibration Procedure

· Calibration must be performed using a calibrator of the same lot number as the reagent pack.
· Refer to the VITROS ECi/ECiQ Immunodiagnostic 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-HBc 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 when to calibrate, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.

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-HBc decision point [signal/cutoff (s/c) ≤1.00].
· 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/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 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 recommendations[11].

· 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 a composition similar to or identical with the patient sample matrix being analyzed[12]. VITROS Anti-HBc Controls are recommended for use with the VITROS ECi/ECiQ Immunodiagnostic 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 must be established for all commercially available quality control materials used with the VITROS Anti-HBc 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

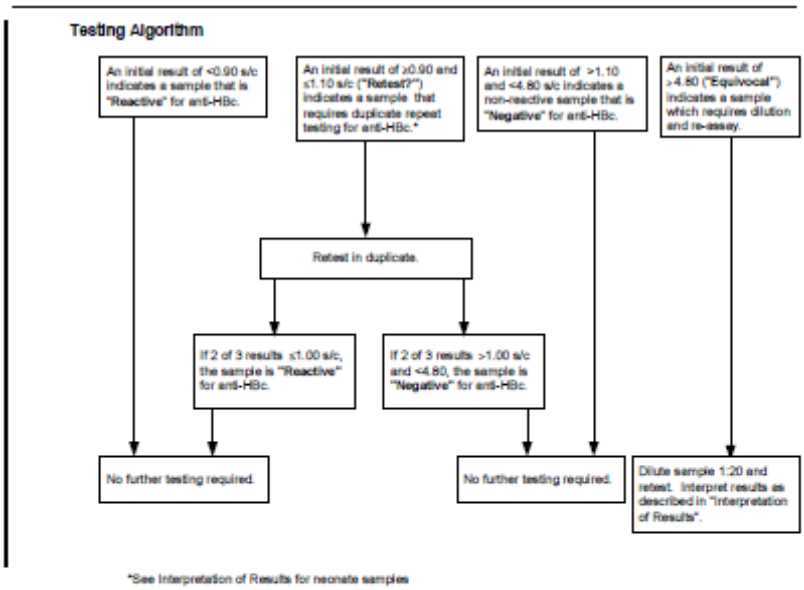
Results are calculated as a normalized signal, relative to the cutoff value (signal/cutoff, s/c). During the calibration process, a lot-specific parameter, encoded on the lot calibration card, is used to determine a valid stored cutoff value for the VITROS ECi/ECiQ Immunodiagnostic System.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

Patient sample results will be displayed with a **"Reactive"**, **"Retest?", "Negative", or "Equivocal"** label. An initial result labeled with **"Retest?"** indicates a sample that requires duplicate repeat testing for anti-HBc. An initial result labeled "**Equivocal**" indicates a sample which requires dilution and re-assay.

Result (s/c)	<0.90	≥0.90 and ≤1.10	>1.10 and <4.80	≥4.80
Result Text	**Reactive**	**Retest?**	**Negative**	**Equivocal**

Final results should be manually interpreted using the algorithm below.



Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS Anti-HBc assay.

Initial VITROS Anti-HBc Assay Result (s/c)	Conclusion from Testing Algorithm	Interpretation
<0.90	Reactive	Specimen is presumed to be reactive for anti-HBc.
≥0.90 and ≤1.10	Retest in duplicate	If 2 of 3 results are ≤1.00 then specimen is presumed to be reactive for anti-HBc.
≥0.90 and ≤1.10	Retest in duplicate	If 2 of 3 results are >1.00 and <4.80 then specimen is negative for anti-HBc.
>1.10 and <4.80	Negative	Specimen is negative for anti-HBc.
≥4.80	Dilute 1:20 and retest	If 1:20 dilution and retest[†] result is ≤1.00, then specimen is presumed to be reactive for anti-HBc.
≥4.80	Dilute 1:20 and retest	If 1:20 dilution and retest[†] result is >1.00 and <4.80, then specimen is negative for anti-HBc.

[†]Results of the diluted sample do not require correction for the dilution factor.

- Results obtained with the VITROS Anti-HBc assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

· The magnitude of a VITROS Anti-HBc assay result cannot be correlated to an endpoint titer.
· Neonate samples with results ≥0.90 and ≤1.10 should not be retested in duplicate. Obtain a new sample and retest.

Expected Results

Approximately 65.7% (1111/1691) of the prospective subjects in Population I reported no recent or current signs or symptoms of hepatitis. Of these 1111 asymptomatic individuals, 25.3% were enrolled in Miami, FL, 36.5% were enrolled in Dallas, TX, and 38.2% were enrolled in Chicago, IL. The group was Caucasian (27.7%), African American (44.8%), Hispanic (18.7%), and Asian (4.8%) with the remaining 4% represented by other ethnic groups. The group was 51.6% male and 48.4% female and ranged in age from 5 to 89 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS Anti-HBc assay was reactive in 20.6% of the individuals in this group. The percent VITROS Anti-HBc reactive results observed in the asymptomatic population at each site was 4.5% at Miami, FL, 8.3% at Dallas, TX, and 7.8% at Chicago, IL.

The table below summarizes the distribution of VITROS Anti-HBc reactive and negative results among the study subjects without signs or symptoms of hepatitis, by age and gender.

Expected Results for the VITROS Anti-HBc Assay in Study Subjects Without Signs or Symptoms of Hepatitis - Population I (N=1111)						
Age Range	Gender	VITROS Anti-HBc Result				
		Reactive		Negative		Total
		N	Percent	N	Percent	
0–9	Female	0	0	0	0	0
	Male	0	0	1	100	1
10–19	Female	3	27.3	8	72.7	11
	Male	0	0	11	100	11
20–29	Female	3	3.1	93	96.9	96
	Male	4	4.8	80	95.2	84
30–39	Female	14	12.0	103	88.0	117
	Male	39	24.2	122	75.8	161
40–49	Female	30	23.3	99	76.7	129
	Male	63	35.0	117	65.0	180
50–59	Female	13	14.0	80	86.0	93
	Male	19	27.1	51	72.9	70
60–69	Female	15	23.8	48	76.2	63
	Male	11	27.5	29	72.5	40
70–79	Female	5	22.7	17	77.3	22
	Male	7	30.4	16	69.6	23
80–89	Female	2	40.0	3	60.0	5
	Male	1	50.0	1	50.0	2
90–100	Female	0	0	0	0	0
	Male	0	0	0	0	0
Total		229	20.7	879	79.3	1108*

* Age was not reported for 3 subjects.

Expected results for the VITROS Anti-HBc assay were also determined using unlinked samples from a population of pediatric and adolescent subjects in Utah at low risk for viral hepatitis (N=100). The group was 57% male and 43% female, and the subjects' ages ranged from two to 19 years. Three (3.0%) samples were reactive with the VITROS Anti-HBc assay and were tested with the reference anti-HBc assay. Two of these three were found to be reference anti-HBc assay reactive.

Expected Results for the VITROS Anti-HBc Assay in Pediatric and Adolescent Subjects At Low Risk for Viral Hepatitis (N=100)						
Age Range	Gender	VITROS Anti-HBc Result				
		Reactive		Negative		Total
		N	Percent	N	Percent	
2–4	Female	0	0	9	100	9
	Male	0	0	16	100	16
5–9	Female	0	0	13	100	13
	Male	1	8.3	11	91.7	12
10–14	Female	0	0	8	100	8
	Male	1	5.9	16	94.1	17
15–19	Female	1	7.7	12	92.3	13
	Male	0	0	12	100	12
Total		3	3.0	97	97.0	100

Limitations of the Procedure

· The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to hepatitis B virus. Levels of anti-HBc may be undetectable both in early infection and late after infection.

· Heterophilic antibodies in serum or plasma samples may cause interference with immunoassays[13]. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results, which are inconsistent with clinical observations indicate the need for additional testing.

Performance Characteristics

Clinical Performance

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

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

The subjects in Population I were Caucasian (24.9%), African American (44.1%), Hispanic (22.4%) and Asian (3.7%), with the remaining 4.9% represented by other ethnic groups. The group was 52.4% male and 47.6% female, and ranged in age from 5 to 89 years. Testing of these specimens with the VITROS Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (37.0%), Port Jefferson, NY (34.9%) and Minneapolis MN, (28.1%). Agreement of the VITROS Anti-HBc assay was assessed relative to the reference anti-HBc assay and HBV disease classification using serum samples from the 1691 subjects in Population I.
The subjects in Population II were Indian (100.0%). The group was 73.0% male and 27.0% female, and ranged in age from 18 to 90 years. Testing of these specimens with the VITROS Anti-HBc assay occurred at diagnostic laboratories located in Miami, FL (33.0%), Minneapolis MN, (32.4%) and Los Angeles, CA (34.6%). Agreement of the VITROS Anti-HBc assay was assessed relative to the reference anti-HBc assay and HBV disease classification using serum samples from the 315 subjectsin Population II.

Results by Specimen Classification

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

HBV Reference Marker Profiles and HBV Disease Classification

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

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

Comparison of Results

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

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

HBV Disease Classification	Reference Anti-HBc Result				
	Reactive		Negative		
	VITROS Anti-HBc Result		VITROS Anti-HBc Result		
	Reactive	Negative*	Reactive	Negative	Total
Acute	8	0	0	9	17
Chronic	40	3	0	0	43
Early Recovery	46	1	0	0	47
Recovery	138	0	0	0	138
Recovered	168	28	0	0	196
HBV Vaccine Response	0	0	0	189	189
Not Previously Infected with HBV	0	0	5**	1069	1074
Uninterpretable	0	1	0	6	7
Overall	400	33	5	1253	1691

*These samples were tested with a second FDA approved anti-HBc assay with the following results:
Chronic: 2/3 negative,
Early recovery: 1/1 indeterminate,
Recovered: 18/28 negative; 1/28 indeterminate,
Uninterpretable: 1/1 negative,
Overall: 23/33 (69.7%) negative or indeterminate.
**These samples were tested with a second FDA approved anti-HBc assay with the following results: 2/5 (40%) positive.

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

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

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

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

Percent Agreement

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

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

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

The positive percent agreement with the reference anti-HBc assay was determined by dividing the number of reactive VITROS Anti-HBc results by the total number of subjects reactive with the reference anti-HBc assay. As a result of this study, the overall positive percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population I was estimated to be 92.38% (400/433, with a 95% exact confidence interval of 89.46% to 94.70%).
The negative percent agreement with the reference anti-HBc assay was determined by dividing the number of negative VITROS Anti-HBc results by the total number of subjects negative with the reference anti-HBc assay. As a result of this study, the overall negative percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population I was estimated to be 99.60% (1253/1258, with a 95% exact confidence interval of 99.07% to 99.87%).

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

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

The positive percent agreement with the reference anti-HBc assay was determined by dividing the number of reactive VITROS Anti-HBc results by the total number of subjects repeatedly reactive with the reference anti-HBc assay. As a result of this study, the overall positive percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 98.56% (273/277, with a 95% exact confidence interval of 96.34% to 99.61%).
The negative percent agreement with the reference anti-HBc assay was determined by dividing the number of negative VITROS Anti-HBc results by the total number of subjects negative with the reference anti-HBc assay. As a result of this study, the overall negative percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay in Population II was estimated to be 100.0% (38/38, with a 95% exact confidence interval of 90.75% to 100.0%).
The performance of the VITROS Anti-HBc assay was further evaluated among archived serum samples from subjects with clinical and laboratory documentation of acute or chronic (HBsAg present for ≥6 months) HBV infection. The table below summarizes the performance of the VITROS Anti-HBc assay in samples from subjects with documented acute or chronic HBV infection.

Overall Clinical Performance of the VITROS Anti-HBc Assay in Samples from Subjects with Clinically Documented Acute or Chronic HBV Infection			
HBV Infection	Number of Samples	Number (%) of VITROS Anti-HBc Reactive Samples	95% Exact Confidence Interval
Acute	8	8 (100.0)	63.06 – 100.0
Chronic	76	75 (98.7)	92.89 – 99.97
Total	84	83 (98.8)	93.54 – 99.97

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

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

VITROS and Reference Anti-HBc Results in Pre-Vaccination Samples (N=41)		
Test Result	Reference Anti-HBc Assay	VITROS Anti-HBc Assay
Initially Negative	37	41
Initially Reactive	4	NA
Repeatedly Reactive	0	NA
Total Negative Results	41	41

Seroconversion Panels

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

Anti-HBc Seroconversion Panel Study - Summary Results					
Days to Reactive Anti-HBc Result					
	Reference Anti-HBc Assay		VITROS Anti-HBc Assay		Difference in Days to Anti-HBc Reactive Result
Panel ID	–*	+**	–*	+**	Reference - VITROS
6278	28	33	33	37	-4
6281	38	41	38	41	0
PHM935A	50	66	50	66	0
RP009	13	29	13	29	0
RP016	24	56	24	56	0
RP017	43	65	43	65	0

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

Potentially Cross-Reacting Subgroups

Samples with evidence of hepatitis A virus infection (HAV) or hepatitis C virus infection (HCV) were identified in a population of 1691 samples prospectively collected from subjects in the U.S with signs or symptoms of, or at risk for, viral hepatitis

(Population I). The tables below compare VITROS Anti-HBc results with reference anti-HBc results according to the HBV disease classifications assigned to the study subjects.

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

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

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

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

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

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

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

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

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

The specificity of the VITROS Anti-HBc assay was evaluated by testing 232 samples from 16 potentially cross-reacting subgroups. Patient samples from the following sub-groups were tested: HAV, HEV, HCV, non-viral liver disease, autoimmune disease (rheumatoid arthritis and systemic lupus erythrematosis), CMV, EBV, HSV, parvovirus B19 infection, rubella, syphilis, toxoplasmosis, HIV 1/2 antibody positive, HTLV 1/2 antibody positive, and HBV vaccine recipients.
Of the 232 samples tested, 230 were observed to be negative. One autoimmune disease (rheumatoid arthritis) sample was

initially reactive in the VITROS Anti-HBc assay, but was negative on repeat determination. One Syphilis sample was reactive initially in the VITROS Anti-HBc assay and also on repeat determination.

Summary of Specificity Data from Cross-Reacting Sub-Groups				
Sample Category	No. Samples Tested	VITROS Negative	VITROS Initial Reactive	VITROS Repeat Reactive
Hepatitis A Infection	14	14	0	0
Hepatitis C Infection	10	10	0	0
HEV Infection	5	5	0	0
Nonviral Liver Disease	50	50	0	0
Autoimmune Diseases (Rheumatoid arthritis)	50	49	1	0
Autoimmune Diseases (Lupus Erythrematosis)	10	10	0	0
CMV IgM Positive	10	10	0	0
EBV IgM Positive	10	10	0	0
HSV IgM Positive	10	10	0	0
Parvovirus B19 Infection	10	10	0	0
Rubella Infection	10	10	0	0
Syphilis Infection	10	9	1	1
Toxoplasmosis Infection	10	10	0	0
HIV ½ Ab Positive	5	5	0	0
HTLV ½ Ab Positive	9	9	0	0
HBV Vaccine Recipients	9	9	0	0

A total of 20 cord blood patient samples were tested in the VITROS Anti-HBc assay.
In testing the cord blood samples, 1 out of 20 samples was found to give a repeatedly reactive result in the VITROS Anti-HBc assay. This repeatedly reactive sample was also repeatedly reactive in the reference method.

Summary of VITROS Anti-HBc Specificity Data from Cross-Reacting Sub-Groups				
Sample Category	No. Samples Tested	No. Negative	No. Initially Reactive	No. Repeatedly Reactive*
Cord Blood	20	19	1	1

* The sample was also repeatedly reactive with the reference Anti-HBc assay.

Substances that do not Interfere

The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 10 blood donors. The results (mean of test results at each level of interferent) demonstrate that hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL) and triolein (up to 3000 mg/dL), cause no misclassification of results. Anti-HBc spiked samples were tested near the cut-off (cut-off s/c=1.00), and were observed to remain reactive at all levels tested with each potential interferent. Similarly no interference was observed in samples not spiked with anti-HBc (Negative), with anti-HBc values remaining above 2.00 s/c.

Test Substance	Maximum Level Tested	Mean Result at 0 Interferent Level		Mean Result at Maximum Interferent Level	
		s/c	Classification	s/c	Classification
Hemoglobin					
anti-HBc Spiked Specimen	500 mg/dL	0.76	Reactive	0.76	Reactive
anti-HBc Negative Specimen	500 mg/dL	2.97	Negative	2.92	Negative
Bilirubin					
anti-HBc Spiked Specimen	20 mg/dL	0.69	Reactive	0.50	Reactive
anti-HBc Negative Specimen	20 mg/dL	2.99	Negative	2.91	Negative
Triolein					
anti-HBc Spiked Specimen	3000 mg/dL	0.62	Reactive	0.54	Reactive
anti-HBc Negative Specimen	3000 mg/dL	2.98	Negative	3.00	Negative

Precision

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

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

* Within Day: Variability of the assay performance from replicate to replicate.
† Between 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 three replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates (CV (%)) were derived from a variance component analysis. The data shown in the table were rounded following all calculations.

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

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

Warning: ***Assay reproducibility has not been established when using a plasma matrix. It is recommended when using these matrices the user establish reproducibility by performing the appropriate reproducibility studies.***

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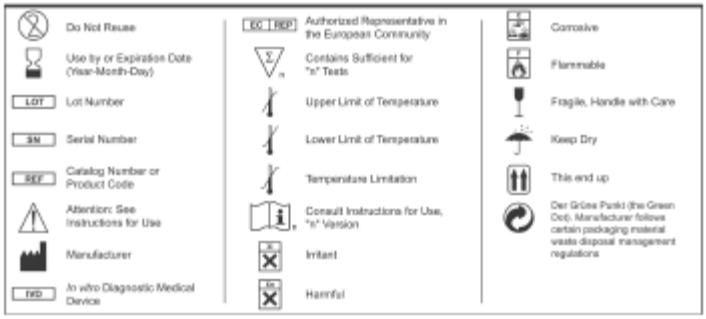
Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Revision History

Date of Revision:	Version:	Description:
2008-03-04	3.0	Formatting updates Updated NCCLS to CLSI Materials Required But Not Provided: Add VITROS Immunodiagnostic Products High Sample Diluent B Assay Procedure: Add Sample Dilution section Interpretation of Results and Expected Results: Section updated to add instrument flag of Equivocal for samples with results ≥4.80. Testing Algorithm: updated to include samples with results of >1.10 and <4.80 and samples with results ≥4.80 Interpretation of Results: In the first sentence removed "upon completion of all testing steps required in the testing algorithm". Updated table to include samples with results of >1.10 and <4.80 and samples with results ≥4.80 References: Updated reference 6
2007-10-04	2.1	Address Block: Added Novartis Vaccines and Diagnostics, Inc. Emeryville, CA 94608-2916
2005-02-03	2.0	Changed all occurrences of "VITROS Immunodiagnostic System", "VITROS ECi System" and "VITROS ECi Immunodiagnostic System" to VITROS ECi/ECiQ Immunodiagnostic System. Testing Algorithm: Modified algorithm for consistency with other hepatitis flowcharts 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.
2004-02-06	1.0	Initial version of Instructions for Use

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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