

# Multi-center Evaluation of the VITROS® Anti-HBc Assay in Patients with Signs and Symptoms of Hepatitis and in Persons at Risk for Hepatitis

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## INTRODUCTION

A multi-center outcomes based study was conducted to evaluate the clinical effectiveness of the VITROS® Anti-HBc assay as an aid in the laboratory diagnosis of individuals with acute or chronic hepatitis B. 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). 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. Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection. The VITROS Anti-HBc assay is performed using the VITROS Anti-HBc Immunodiagnostic Products Reagent Pack and VITROS Immunodiagnostic Products Anti-HBc Calibrator on the VITROS ECi 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. 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-HBc present in the sample.

Two at-risk prospective sample populations were evaluated. Population I (N=1691) was collected in the U.S. from persons with signs or symptoms or biochemical manifestations of hepatitis (elevated liver function tests) and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, or known exposure event. These specimens were obtained from subjects enrolled at three collection sites that were located in Miami, FL (37.0%), Dallas, TX (28.1%) and Chicago, IL (34.9%). These specimens were tested at diagnostic laboratories located in Miami, FL, Port Jefferson, NY, and Minneapolis, MN. Population II (N=315) was obtained from subjects prospectively enrolled from an area in India with a high prevalence of viral hepatitis; all subjects in this population reported signs or symptoms of viral hepatitis. Testing of these specimens was done at diagnostic laboratories located in Miami, FL, Minneapolis, MN, and Los Angeles, CA. The demographic profiles of these prospective study populations are shown in Tables 1 and 2. These results are listed by testing site rather than by collection site. A third, unlinked population of samples (N=100) from a population of pediatric and adolescent subjects in Utah at low risk for viral hepatitis was also tested with the VITROS Anti-HBc assay.

**Table 1: Demographic Profiles of the Prospective Study Subjects in Population I**

	Miami, FL N (%)	Port Jefferson, NY N (%)	Minneapolis, MN N (%)	TOTAL N (%)
<b>Test Site</b>				
TOTAL	626 (37.0)	590 (34.9)	475 (28.1)	1691 (100.0)
<b>GENDER</b>				
Male	321	289	276	886 (52.4)
Female	305	301	199	805 (47.6)
<b>ETHNICITY</b>				
Caucasian	182	59	180	421 (24.9)
African-American	135	374	237	746 (44.1)
Hispanic	217	111	51	379 (22.4)
Asian	34	27	1	62 (3.7)
Indian	0	15	4	19 (1.1)
Haitian	32	0	0	32 (1.9)
Other	11	3	2	16 (0.9)
Unknown	15	1	0	16 (0.9)
<b>AGE</b>				
≤10	1	0	0	1 (0.1)
11-30	37	137	133	307 (18.2)
31-50	256	311	295	862 (51.0)
51-70	279	126	45	450 (26.6)
>70	48	16	2	66 (3.9)
Unknown	5	0	0	5 (0.3)
<b>RISK</b>				
No Risk Factor(s)	97	17	15	129 (7.6)
Risk Factor(s)	529	573	460	1562 (92.4)

**Table 2: Demographic Profiles of the Prospective Study Subjects in Population II**

	Miami, FL N (%)	Minneapolis, MN N (%)	Los Angeles, CA N (%)	TOTAL N (%)
<b>Test Site</b>				
TOTAL	104 (33.0)	102 (32.4)	109 (34.6)	315 (100.0)
<b>GENDER</b>				
Male	76	70	84	230 (73.0)
Female	28	32	25	85 (27.0)
<b>AGE</b>				
11-30	33	29	50	112 (35.6)
31-50	57	62	42	161 (51.1)
51-70	13	11	17	41 (13.0)
>70	1	0	0	1 (0.3)

## STUDY DESIGN AND HBV DISEASE CLASSIFICATION

The HBV disease classification of each study subject was assigned by using a hepatitis B marker profile that consisted of reference assays (previously licensed or approved by the FDA) for the detection of HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, and anti-HBs (quantitative). All reference assays were from one manufacturer with the exception of the HBeAg and anti-HBe assays used to test samples from Population II. The positive (+) / negative (-) results from this reference assay testing were used to assign each sample/subject to an HBV disease classification. Table 3 summarizes how these classifications were determined. There were 28 distinct reference marker profiles

observed among the subjects in Populations I and II. Both populations demonstrated 14 profiles in common; 10 profiles were observed only in Population I and 4 profiles were found only in Population II. Six of the 28 profiles did not allow assignment to any of the recognized HBV disease classifications and are reported as “uninterpretable.”

**Table 3: HBV Reference Marker Profiles and HBV Disease Classification**

HBsAg*	HBeAg	IgM aHBc	Total aHBc	aHBe	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 HBV-Previously Infected
+	+	-	-	+	+	Uninterpretable
+	-	-	-	-	+	Uninterpretable
-	+	-	+	-	-	Uninterpretable
-	+	-	-	-	+	Uninterpretable
-	+	-	-	-	-	Uninterpretable
-	-	+	-	-	-	Uninterpretable

\* (+): Reference HBsAg assay reactive and confirmed by neutralization; (-): Reference HBsAg assay negative or not confirmed by neutralization

## COMPARISON OF RESULTS IN AT-RISK PROSPECTIVE POPULATIONS

Table 4 compares the results of the VITROS Anti-HBc assay with the results of the reference anti-HBc assay by specimen classification in prospective Population I.

**Table 4: 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				Total
	Reactive		Negative		
	VITROS Anti-HBc Result Reactive	VITROS Anti-HBc Result Negative	VITROS Anti-HBc Result Reactive	VITROS Anti-HBc Result Negative	
Overall	400	33	5	1253	1691
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	169	169
Not Previously Infected with HBV	0	0	5	1069	1074
Uninterpretable	0	1	0	6	7

Table 5 summarizes the percent agreement between the VITROS and reference anti-HBc assays for each specimen classification in prospective sample Population I. Percent agreement was determined by dividing the number of positive or negative VITROS Anti-HBc assay results by the number of positive or negative reference anti-HBc assay results, respectively.

**Table 5: Positive and Negative Percent Agreement between the VITROS Anti-HBc and Reference Anti-HBc Assays in Population I (N=1691)**

HBV Disease Classification	Positive Agreement N (%)	95% Exact Confidence Interval	Negative Agreement N (%)	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

Table 6 compares the results of the VITROS Anti-HBc assay with the results of the reference anti-HBc assay by specimen classification in prospective Population II.

**Table 6: 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				Total
	Reactive		Negative		
	VITROS Anti-HBc Result Reactive	VITROS Anti-HBc Result Negative	VITROS Anti-HBc Result Reactive	VITROS Anti-HBc Result Negative	
Overall	273	4	0	38	315
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

Table 7 summarizes the percent agreement between the VITROS and reference anti-HBc assays for each specimen classification in prospective sample Population II.

**Table 7: Positive and Negative Percent Agreement between the VITROS Anti-HBc and Reference Anti-HBc Assays in Population II (N=315)**

HBV Disease Classification	Positive Agreement N (%)	95% Exact Confidence Interval	Negative Agreement N (%)	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

## PRESUMABLY VITROS FALSE NEGATIVE RESULTS

Within Populations I & II combined, 37 samples gave a positive anti-HBc result with the reference assay (initial S/C  $\leq 1.0$  and at least one of two repeat test results  $\leq 1.0$ ) and a negative result with the VITROS Anti-HBc assay. With 12 (32%) of these samples the reference anti-HBc result was the only positive HBV marker. Table 8 summarizes the details for these 12 samples. At the completion of this study, these 12 samples were tested at an independent reference laboratory by a second FDA approved assay. These independent testing results are shown in Table 8 also.

**Table 8: Results for Presumably False Negative VITROS Anti-HBc Samples with Anti-HBc as the Only Positive HBV Reference Marker**

VITROS Result Total aHBc Median S/C (Interpretation)	VITROS Disease Classification	Reference Test Result Total aHBc Median S/C (Interpretation)	Reference Test Disease Classification	Second Assay Test Result	Revised Disease Classification
3.41 (-)	NPI*	0.99 (+)	Recovered	-	NPI
1.56 (-)	NPI	0.95 (+)	Recovered	-	NPI
1.38 (-)	NPI	0.65 (+)	Recovered	-	NPI
1.04 (-)	NPI	0.30 (+)	Recovered	+	Recovered
1.63 (-)	NPI	0.88 (+)	Recovered	-	NPI
1.23 (-)	NPI	0.64 (+)	Recovered	IND**	Unknown
1.48 (-)	NPI	0.78 (+)	Recovered	-	NPI
1.99 (-)	NPI	0.74 (+)	Recovered	-	NPI
1.08 (-)	NPI	0.64 (+)	Recovered	+	Recovered
3.52 (-)	NPI	0.71 (+)	Recovered	-	NPI
1.30 (-)	NPI	0.70 (+)	Recovered	-	NPI
2.49 (-)	NPI	1.00 (+)	Recovered	+	Recovered

\* Not Previously Infected with HBV

\*\* Indeterminate

Of the 12 samples initially classified as Recovered on the basis of the reference test anti-HBc results and classified as Not Previously Infected with HBV by the VITROS anti-HBc result, 8/12 (67%) were also classified as Not Previously Infected according to the results of another FDA approved assay.

With 17 samples (46%) which gave disagreeing results between the VITROS and the reference anti-HBc assays, anti-HBc and anti-HBs were the only reference-assay positive HBV markers. Table 9 summarizes the details for these 17 samples. The results of testing with a second FDA approved assay are shown also.

**Table 9: Results for Presumably False Negative VITROS Anti-HBc Samples with Anti-HBc and Anti-HBs as the Only Positive Reference Markers**

VITROS Result Total aHBc Median S/C (Interpretation)	VITROS Disease Classification	Reference Test Result Total aHBc Median S/C (Interpretation)	Reference Test Disease Classification	Second Assay Test Result	Revised Disease Classification
1.07 (-)	VR*	0.76 (+)	Recovered	+	Recovered
1.57 (-)	VR	0.82 (+)	Recovered	-	VR
1.23 (-)	VR	0.39 (+)	Recovered	+	Recovered
2.31 (-)	VR	0.90 (+)	Recovered	-	VR
1.21 (-)	VR	0.39 (+)	Recovered	+	Recovered
1.09 (-)	VR	0.67 (+)	Recovered	-	VR
1.39 (-)	VR	0.93 (+)	Recovered	-	VR
1.55 (-)	VR	0.64 (+)	Recovered	+	Recovered
2.8 (-)	VR	0.75 (+)	Recovered	-	VR
1.88 (-)	VR	0.56 (+)	Recovered	-	VR
1.40 (-)	VR	0.75 (+)	Recovered	-	VR
1.42 (-)	VR	0.70 (+)	Recovered	-	VR
1.01 (-)	VR	0.40 (+)	Recovered	-	VR
1.24 (-)	VR	0.57 (+)	Recovered	+	Recovered
1.74 (-)	VR	0.73 (+)	Recovered	-	VR
1.52 (-)	VR	0.31 (+)	Recovered	+	Recovered
1.41 (-)	VR	0.39 (+)	Recovered	+	Recovered

\* Vaccine Responder

Of the 17 samples initially classified as Recovered on the basis of the reference test anti-HBc results and classified as Vaccine Responders by the VITROS anti-HBc result, 10/17 (59%) were also classified as Vaccine Responders according to the results of another FDA approved assay.

The remaining 8 of the 37 presumably VITROS Anti-HBc false negative samples were classified as: acute (N=2), chronic (N=4), early recovery (N=1), and uninterpretable (N=1). The complete marker profiles for these eight samples are shown in Table 10.

**Table 10: HBV Reference Marker Profiles and HBV Disease Classification for Presumably False Negative VITROS Anti-HBc Samples with Multiple Positive Reference Markers**

Reference HBsAg	Reference HBeAg	Reference IgM aHBc	Reference Total aHBc	Reference aHBe	Reference aHBs	HBV Disease Classification
+	-	+	+	-	-	Acute
+	-	+	+	+	-	Acute
+	+	-	+	-	-	Chronic
+	+	-	+	-	-	Chronic
+	-	-	+	-	-	Chronic
+	-	-	+	+	-	Chronic
-	-	-	+	+	-	Early Recovery
-	+	-	+	-	-	Uninterpretable

Table 11 summarizes the details for these 8 samples. The results of testing with a second FDA approved assay are shown also.

**Table 11: Results for Presumably False Negative VITROS Anti-HBc Samples with Multiple Positive Reference Markers**

VITROS Result Total aHBc Median S/C (Interpretation)	VITROS Disease Classification	Reference Test Result Total aHBc Median S/C (Interpretation)	Reference Test Disease Classification	Second Assay Test Result
1.32 (-)	Acute	0.04 (+)	Acute	+
1.26 (-)	Acute	0.06 (+)	Acute	+
3.29 (-)	Chronic	0.15 (+)	Chronic	-
1.67 (-)	Chronic	0.24 (+)	Chronic	+
3.03 (-)	Chronic	0.58 (+)	Chronic	-
1.06 (-)	Chronic	0.09 (+)	Chronic	QNS*
1.35 (-)	Early Recovery	0.33 (+)	Early Recovery	Indeterminate
3.31 (-)	Uninterpretable	0.60 (+)	Uninterpretable	-

\*Insufficient sample volume for additional testing

## PRESUMABLY VITROS FALSE POSITIVE RESULTS

Five samples (all from Population I), gave a positive anti-HBc result with the VITROS Anti-HBc assay and a negative result with the reference assay. Table 12 summarizes the details for these five samples. At the completion of this study, these samples were tested at an independent reference laboratory by a second FDA approved assay. These independent testing results are shown in Table 12 also.

**Table 12: Results for Presumably False Positive VITROS Anti-HBc Samples**

VITROS Result Total aHBc Median S/C (Interpretation)	Reference Test Result Total aHBc Median S/C (Interpretation)	HBsAg	HBeAg	IgM aHBc	Total aHBc	aHBe	aHBs ≥10 mIU/mL	Second Assay Test Result
0.97 (+)	1.63 (-)	-	-	-	-	-	-	-
0.89 (+)	1.38 (-)	-	-	-	-	-	-	-
0.92 (+)	1.32 (-)	-	-	-	-	-	-	-
0.04 (+)	1.75 (-)	-	-	-	-	-	-	+
0.04 (+)	3.28 (-)	-	-	-	-	-	-	+

## RESULTS OF TESTING A LOW RISK POPULATION

The performance of the VITROS Anti-HBc assay was further evaluated by testing 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. The demographic profile of this population, and the testing results are shown in Table 13.

**Table 13: 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				Total
		Reactive		Negative		
		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

Three samples (3.0%) were reactive with the VITROS Anti-HBc assay and were tested with the reference anti-HBc assay. Of the three samples that were reactive in the VITROS Anti-HBc assay, two of these were found to be reactive also with the reference anti-HBc assay.

## RESULTS OF TESTING ARCHIVED SAMPLES

The performance of the VITROS Anti-HBc assay was further evaluated by testing archived serum samples from subjects with clinical and laboratory documentation of acute or chronic (HBsAg present for ≥6 months) HBV infection. Table 14 summarizes the performance of the VITROS Anti-HBc assay when used in this sample population.

**Table 14: 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 Disease
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

Serum samples obtained from 41 individuals immediately prior to HBV vaccination were tested with the VITROS and reference anti-HBc assays. The results for both assays are shown in Table 15.

**Table 15: 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	0
Repeatedly Reactive	0	NA
Total Negative Results	41	41

## RESULTS OF TESTING SEROCONVERSION PANELS

Six commercially available seroconversion panels were tested. The VITROS and reference anti-HBc assay results are summarized below. Table 16 lists the first bleed of each panel that tested reactive with the VITROS and the reference assays as well as the difference in days between the two assays in identifying the first reactive panel member.

**Table 16: Anti-HBc Seroconversion Panel Study - Summary Results**

Panel ID	Days to Reactive Anti-HBc Result				Difference in Days to Anti-HBc Reactive Result
	Reference Anti-HBc Assay		VITROS Anti-HBc Assay		
	-*	+**	-*	+**	Reference - VITROS
6278	26	33	33	37	-4
6281	36	41	36	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

\*Days from the initial bleed day (Day 0) until last nonreactive result, usually denotes the bleed just before the first reactive result

\*\*Days from the initial bleed day (Day 0) until first reactive result

## RESULTS OF TESTING POTENTIALLY CROSS-REACTING SUBGROUPS

Samples with evidence of hepatitis A virus infection (HAV) and/or hepatitis C virus infection (HCV) were identified in the 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). Tables 17 and 18 compare the VITROS and reference anti-HBc results according to the HBV disease classifications assigned to the study subjects. Any non-agreeing results observed between the VITROS Anti-HBc assay and the reference anti-HBc assay have been reviewed in prior sections of this document.

**Table 17: 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 VITROS Anti-HBc Result		Negative VITROS Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	
Overall	2	0	0	5	7
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

**Table 18: 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 VITROS Anti-HBc Result		Negative VITROS Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	
Overall	169	10	1	173	353
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

Samples with evidence of hepatitis A virus infection (HAV) and/or hepatitis C virus infection (HCV) were also identified in the population of 315 samples prospectively collected in India (Population II). Tables 19 and 20 compare the VITROS and reference anti-HBc results, by HBV disease classifications, for subjects with evidence of HAV or HCV infection, respectively. Any non-agreeing results observed between the VITROS Anti-HBc assay and the reference anti-HBc assay have been reviewed in prior sections of this document.

**Table 19: 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 VITROS Anti-HBc Result		Negative VITROS Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	
Overall	10	2	0	17	29
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

**Table 20: 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 VITROS Anti-HBc Result		Negative VITROS Anti-HBc Result		
	Reactive	Negative	Reactive	Negative	
Overall	90	0	0	0	90
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

## SUMMARY AND CONCLUSIONS

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). Specimens were also obtained from 315 subjects prospectively enrolled from an area in India with a high prevalence of viral hepatitis (Population II). 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, IgM anti-HBc, 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, Port Jefferson, NY, and Minneapolis MN. 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, Minneapolis MN, and Los Angeles, CA.

The positive and negative agreements of the VITROS and reference anti-HBc assays were determined for the individual HBV disease classification and for the prospective populations overall. 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 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%). In this study, in Population II, the overall positive percent agreement of the VITROS Anti-HBc assay with the reference anti-HBc assay was estimated to be 98.56% (273/277, with a 95% exact confidence interval of 96.34% to 99.61%). 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%).

Of the 37 presumably false negative VITROS anti-HBc results (VITROS anti-HBc negative and reference anti-HBc assay reactive) observed in prospective Populations I and II, 36 were available in sufficient volume to be tested with a second FDA approved anti-HBc assay. This second assay gave 21 negative results and 13 positive results (2 samples gave indeterminate results). Of the five samples from Population I that demonstrated presumably VITROS anti-HBc false positive results, two samples gave positive results with this second FDA approved assay also.

Unlinked samples from a population of pediatric and adolescent subjects at low risk for viral hepatitis (N=100) were also tested with the VITROS Anti-HBc assay. Of these, 3 samples were reactive, and 97 were negative. Two of the three reactive samples were also reactive when tested with the reference anti-HBc assay.

With samples from individuals with documented acute or chronic HBV infection, the VITROS Anti-HBc assay was reactive with 83 of 84 samples (98.8%, 95% exact confidence interval of 93.54% to 99.97%). The VITROS Anti-HBc assay detected the presence of anti-HBc at the same bleed date as the reference anti-HBc assay in six of seven commercial seroconversion panels. With one panel, the reference anti-HBc assay detected anti-HBc one bleed earlier (equivalent to four days or less) than the VITROS Anti-HBc assay.

Based upon this clinical study, results from the VITROS Anti-HBc assay, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B.

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*The VITROS Anti-HBc assay has been approved by the FDA.*