

Multi-center Evaluation of the VITROS® Anti-HBc IgM 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 IgM assay as an aid in the laboratory diagnosis of individuals with acute or chronic hepatitis B. The VITROS Anti-HBc IgM assay is designed to detect anti-HBc IgM at an appropriate level of sensitivity as an aid for the diagnosis of acute or chronic hepatitis B infection. The detection of anti-HBc IgM can be useful for the differential diagnosis of hepatitis B from other forms of viral hepatitis. IgM class antibodies against HBc are detected soon after infection and the presence of high concentrations of anti-HBc IgM has been shown to be an indicative marker of acute infection. The level of anti-HBc IgM decreases throughout the course of infection. However, low levels of anti-HBc IgM may persist for over a year after infection in some patients and are found occasionally in chronic carriers. The VITROS Anti-HBc IgM assay is performed using the VITROS Anti-HBc IgM Reagent Pack and the VITROS Immunodiagnostic Products Anti-HBc IgM Calibrator on the VITROS ECi Immunodiagnostic System. An antibody class capture technique is used. This involves the dilution of the sample and the simultaneous reaction of IgM in the diluted sample with biotinylated mouse monoclonal anti-human IgM antibody. The immune complex is captured by streptavidin on the wells. Unbound materials are removed by washing. Horseradish peroxidase (HRP)- labeled mouse monoclonal anti-HBc IgM antibody, which has been complexed with recombinant HBc antigen (conjugate) is then captured by anti-HBc specific IgM bound to the wells. Unbound material 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 IgM 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 IgM assay.

Tables 1 and 2 present the demographic profiles of subjects in Populations I and II, respectively.

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

	Test Site	Miami, FL N (%)	Port Jefferson, NY N (%)	Minneapolis, MN N (%)	TOTAL N (%)
	TOTAL	626 (37.0)	590 (34.9)	475 (28.1)	1691 (100.0)
GENDER	Male	321	289	276	886 (52.4)
	Female	305	301	199	805 (47.6)
ETHNICITY	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)
AGE	≤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)
RISK	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

	Test Site	Miami, FL N (%)	Minneapolis, MN N (%)	Los Angeles, CA N (%)	TOTAL N (%)
	TOTAL	104 (33.0)	102 (32.4)	109 (34.6)	315 (100.0)
GENDER	Male	76	70	84	230 (73.0)
	Female	28	32	25	85 (27.0)
AGE	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, total anti-HBc, IgM 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 IgM assay with the results of the reference anti-HBc IgM assay in prospective Population I.

Table 4: Comparison of VITROS Anti-HBc IgM Results with Reference Anti-HBc IgM Results by HBV Disease Classification - Population I (N=1691)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result Reactive	VITROS Anti-HBc IgM Result Negative	VITROS Anti-HBc IgM Result Reactive	VITROS Anti-HBc IgM Result Negative	
Overall	12	7	4	1668	1691
Acute	8	0	0	9	17
Chronic	0	0	2	41	43
Early Recovery	4	6	1	36	47
Recovery	0	0	0	138	138
Recovered	0	0	1	195	196
HBV Vaccine Response	0	0	0	169	169
Not Previously Infected with HBV	0	0	0	1074	1074
Uninterpretable	0	1	0	6	7

Table 5 summarizes the percent agreement between the VITROS and reference anti-HBc IgM 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 IgM assay results by the number of positive or negative reference anti-HBc IgM assay results, respectively.

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

HBV Disease Classification	Positive Agreement N (%)	95% Exact Confidence Interval	Negative Agreement N (%)	95% Exact Confidence Interval
Overall	12/19 (63.16%)	38.36 - 83.71	1668/1672 (99.76%)	99.39 - 99.93
Acute	8/8 (100%)	63.06 - 100	9/9 (100%)	66.37 - 100
Chronic	0/0 (N/A)	N/A	41/43 (95.35%)	84.19 - 99.43
Early Recovery	4/10 (40%)	12.16 - 73.76	36/37 (97.3%)	85.84 - 99.93
Recovery	0/0 (N/A)	N/A	138/138 (100%)	97.36 - 100
Recovered	0/0 (N/A)	N/A	195/196 (99.49%)	97.19 - 99.99
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	1074/1074 (100%)	99.66 - 100
Uninterpretable	0/1 (0%)	N/A	6/6 (100%)	54.07 - 100

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

Table 6: Comparison of VITROS Anti-HBc IgM Results with Reference Anti-HBc IgM Results by HBV Disease Classification - Population II (N=315)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result Reactive	VITROS Anti-HBc IgM Result Negative	VITROS Anti-HBc IgM Result Reactive	VITROS Anti-HBc IgM Result Negative	
Overall	70	19	0	226	315
Acute	69	19	0	16	104
Chronic	0	0	0	185	185
Early Recovery	1	0	0	0	1
Recovery	0	0	0	0	0
Recovered	0	0	0	3	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 IgM assays for each specimen classification in prospective sample Population II.

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

HBV Disease Classification	Positive Agreement (%)	95% Exact Confidence Interval	Negative Agreement N (%)	95% Exact Confidence Interval
Overall	70/89 (78.65%)	68.69 - 86.63	226/226 (100%)	98.38 - 100
Acute	69/88 (78.41%)	68.35 - 86.47	16/16 (100%)	79.41 - 100
Chronic	0/0 (N/A)	N/A	185/185 (100%)	98.03 - 100
Early Recovery	1/1 (100%)	2.5 - 100	0/0 (N/A)	N/A
Recovered	0/0 (N/A)	N/A	3/3 (100%)	29.24 - 100
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

In prospective Populations I and II combined, 26 samples gave negative results with the VITROS Anti-HBc IgM assay and reactive results with the reference anti-HBc IgM assay. Detailed information for these samples is shown in Table 8.

Table 8: Results for Presumably False Negative Vitros Anti-HBc IgM Samples

VITROS Result Anti-HBc IgM Median S/C	Reference Test Result Anti-HBc IgM Median S/C	HBsAg	HBeAg	IgM aHBc	Total aHBc	aHBe	aHBs ≥10 mIU/mL	HBV Disease Classification
0.83	3.17	+	-	+	+	-	-	Acute
0.25	1.42	+	-	+	+	+	-	Acute
0.66	2.18	+	-	+	+	+	-	Acute
0.52	1.84	+	-	+	+	+	-	Acute
0.28	1.41	+	-	+	+	+	-	Acute
0.53	2.24	+	-	+	+	+	-	Acute
0.39	1.75	+	-	+	+	+	-	Acute
0.55	2.21	+	-	+	+	+	-	Acute
0.48	1.89	+	-	+	+	+	-	Acute
0.43	2.06	+	-	+	+	+	-	Acute
0.52	2.05	+	-	+	+	+	-	Acute
0.94	1.24	+	-	+	+	+	-	Acute
0.12	1.24	+	-	+	+	+	-	Acute
0.08	0.87	+	-	+	+	+	-	Acute
0.08	0.82	+	-	+	+	+	-	Acute
0.05	0.83	+	-	+	+	+	-	Acute
0.79	1.07	-	-	+	+	+	+	Early Recovery
0.06	6.35	-	-	+	+	-	+	Early Recovery
0.67	0.86	-	-	+	+	+	+	Early Recovery
0.59	0.83	-	-	+	+	+	+	Early Recovery
0.68	1.02	-	-	+	+	+	-	Early Recovery
0.92	1.01	-	-	+	+	-	-	Early Recovery
0.04	2.93	-	-	-	-	-	-	Uninterpretable
0.32	1.18	-	-	-	-	-	-	Uninterpretable
0.33	1.17	-	-	-	-	-	-	Uninterpretable
0.26	1.36	-	-	-	-	-	-	Uninterpretable

The 26 samples that gave non-agreeing results between the VITROS and reference anti-HBc IgM assays were further evaluated by testing with a second FDA approved assay. The results of this testing and the VITROS and reference assay anti-HBc IgM results and the disease classifications are shown in Table 9.

Table 9: Results for Presumably False Negative VITROS Anti-HBc IgM Samples

VITROS Result Anti-HBc IgM	VITROS Disease Classification	Reference Test Result Anti-HBc IgM	Reference Test Disease Classification	Second Assay Test Result	Revised Disease Classification
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	-	Chronic
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	-	Chronic
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	Indeterminate	Unknown
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	Indeterminate	Unknown
-	Chronic	+	Acute	Indeterminate	Unknown
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	+	Acute
-	Chronic	+	Acute	-	Chronic
-	Chronic	+	Acute	-	Chronic
-	Chronic	+	Acute	-	Chronic
-	Recovery	+	Early Recovery	-	Recovery
-	Recovered	+	Early Recovery	+	Early Recovery
-	Recovery	+	Early Recovery	+	Early Recovery
-	Recovery	+	Early Recovery	-	Recovery
-	Early Recovery	+	Early Recovery	+	Early Recovery
-	Recovered	+	Early Recovery	+	Early Recovery
-	NPI*	+	Uninterpretable	-	NPI
-	Chronic	+	Uninterpretable	-	Chronic
-	Chronic	+	Uninterpretable	-	Chronic
-	Chronic	+	Uninterpretable	-	Chronic

* Not previously infected

Sixteen (of 26) of the samples with presumably false negative VITROS Anti-HBc IgM results had HBV disease classifications of acute HBV because of the reactive reference anti-HBc IgM result. A negative anti-HBc IgM result would change the HBV disease classification to chronic HBV. In the absence of a clinical history for these subjects, the actual disease state, acute or chronic HBV infection, cannot be determined. However, neither anti-HBc IgM result, reactive or negative, would affect the laboratory determination that these subjects were HBV infected.

Six of the VITROS Anti-HBc IgM presumably false negative samples had HBV disease classifications of early recovery because of the reactive reference anti-HBc IgM result. A negative anti-HBc IgM result for these samples would cause the HBV disease classification to remain early recovery for one sample, change to recovery for three samples, and to recovered for the remaining two samples.

Finally, four of the VITROS Anti-HBc IgM presumably false negative samples could not be assigned an HBV disease classification based on the six marker reference assay profile ("Uninterpretable"). The reference anti-HBc IgM result was the only positive marker for one sample and might represent a false positive reference anti-HBc IgM result. A negative anti-HBc IgM result would yield an HBV disease classification of not previously infected, and would appear consistent with this subject's normal ALT level and absence of either signs or symptoms of hepatitis. A negative anti-HBc IgM result for the last three samples would change the HBV disease classification to chronic HBV infection.

PRESUMABLY VITROS FALSE POSITIVE RESULTS

Four samples (all from Population I) gave reactive results with the VITROS Anti-HBc IgM assay and negative results with the reference assay. Table 10 lists the pertinent details about these samples

Table 10: Results for Presumably False Positive VITROS Anti-HBc IgM Samples

VITROS Result Anti-HBc IgM Median S/C	Vitros Disease Classification	Reference Assay HBV Marker Results						Reference Assay HBV Disease Classification
		HBsAg	HBcAg	IgM aHBc	Total aHBc	aHBe	aHBs ≥10 mIU/mL	
1.34	Acute	+	+	-	+	-	-	Chronic
3.53	Acute	+	+	-	+	-	-	Chronic
3.56	Early Recovery	-	-	-	+	+	-	Early Recovery
1.14	Early Recovery	-	-	-	+	-	+	Recovered

These four samples were further evaluated by testing with a second FDA approved assay. All four samples were also reactive with this second assay, in agreement with the VITROS results.

RESULTS OF TESTING A LOW RISK POPULATION

The VITROS Anti-HBc IgM assay was further evaluated in an unlinked subject population comprising 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. As shown in Table 11, all 100 samples were negative with the VITROS Anti-HBc IgM assay.

Table 11: Results of Testing Pediatric and Adolescent Subjects At Low Risk for Viral Hepatitis with the VITROS Anti-HBc IgM Assay (N=100)

Age Range	Gender	VITROS Anti-HBc IgM 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	0	0	12	100	12
10-14	Female	0	0	8	100	8
	Male	0	0	17	100	17
15-19	Female	0	0	13	100	13
	Male	0	0	12	100	12
Total		0	0	100	100	100

RESULTS OF TESTING ARCHIVED SAMPLES

A total of 84 archived serum samples from subjects with clinical and laboratory documentation of acute or chronic (HBsAg present for > 6 months) were evaluated with the VITROS Anti-HBc IgM assay. Table 12 lists the results of this testing.

Table 12: Overall Clinical Performance of the VITROS Anti-HBc IgM Assay in Samples from Subjects with Clinically Documented Acute or Chronic HBV Infection

HBV Infection	Number of Samples	Number (%) of VITROS Anti-HBc IgM Reactive Samples	Number (%) of Reference Anti-HBc IgM Reactive Samples
Acute	8	8 (100.0)	8 (100.0)
Chronic	76	11 (14.5)	30 (39.5)

There was 100% agreement between the VITROS and reference assay results when samples from acute HBV infection were tested. With the 76 samples from chronic cases of HBV, however, the VITROS Anti-HBc IgM was reactive with 11 samples while the reference assay was reactive with 30 samples. This percentage of Vitros Anti-HBc IgM reactive results in this group is consistent with the percentage of anti-HBc IgM reactive results reported in the medical literature. There were

no samples that were reactive with VITROS were negative with the reference assay.

RESULTS OF TESTING SEROCONVERSION PANELS

Six commercially available seroconversion panels were tested. The VITROS and reference anti-HBc IgM assay results are summarized below in Table 13. 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.

Table 13: Anti-HBc IgM Seroconversion Panel Study - Summary Results

Panel ID	Days to Reactive Anti-HBc IgM Result				Difference in Days to Anti-HBc IgM Reactive Result Reference - VITROS
	Reference Anti-HBc IgM Assay		VITROS Anti-HBc IgM Assay		
	- *	+ **	- *	+ **	
6278	33	37	37	41	-4
6281	41	43	41	43	0
PHM935A	50	66	66	68	-2
RP009	36	43	36	43	0
RP016	56	59	56	59	0
RP017	76	78	76	78	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

Within prospective Population I (N = 1691) samples with evidence of hepatitis A virus infection (HAV) and/or hepatitis C virus infection (HCV) were identified. Tables 14 and 15 compare VITROS and reference anti-HBc IgM results, by HBV disease classification, for subjects with evidence of HAV or HCV infection, respectively. Any non-agreeing results observed between the VITROS Anti-HBc IgM assay and the reference anti-HBc IgM assay have been reviewed in prior sections of this document.

Table 14: Comparison of VITROS and Reference Anti-HBc IgM Results and HBV Disease Classification Among Anti-HAV IgM Reactive Study Subjects - Population I (N=7)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Assay	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Assay	
Overall	0	0	0	7	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	0	0	0	2	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 15: Comparison of VITROS and Reference Anti-HBc IgM Results and HBV Disease Classification Among Anti-HCV Reactive Study Subjects - Population I (N=353)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	
Overall	2	3	1	347	353
Acute	1	0	0	3	4
Chronic	0	0	0	9	9
Early Recovery	1	3	0	21	25
Recovery	0	0	0	43	43
Recovered	0	0	1	99	100
HBV Vaccine Response	0	0	0	22	22
Not Previously Infected with HBV	0	0	0	148	148
Uninterpretable	0	0	0	2	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 IgM results, by HBV disease classifications, for subjects with evidence of HAV and/or HCV infection, respectively. Any non-agreeing results observed between the VITROS Anti-HBc IgM assay and the reference anti-HBc IgM assay have been reviewed in prior sections of this document.

Table 16: Comparison of VITROS and Reference Anti-HBc IgM Results and HBV Disease Classification Among Anti-HAV IgM Reactive Study Subjects - Population II (N=29)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	
Overall	3	8	0	18	29
Acute	3	8	0	7	18
Chronic	0	0	0	1	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 17: Comparison of VITROS and Reference Anti-HBc IgM Results and HBV Disease Classification Among Anti-HCV Reactive Study Subjects - Population II (N=90)

HBV Disease Classification	Reference Anti-HBc IgM Result				Total
	Reactive		Negative		
	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	VITROS Anti-HBc IgM Result	
Overall	45	13	0	32	90
Acute	45	13	0	0	58
Chronic	0	0	0	32	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 IgM 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, 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 IgM 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 IgM assay was done at diagnostic laboratories located in Miami, FL, Minneapolis MN, and Los Angeles, CA.

Percent agreement between the VITROS Anti-HBc IgM assay and the reference anti-HBc IgM assay were calculated for subjects in Population I (N=1691) for the various HBV disease classifications, and for the study populations overall. As a result of this study, the overall positive percent agreement of the VITROS Anti-HBc IgM assay with the reference anti-HBc IgM assay in Population I was estimated to be 63.16% (12/19, with a 95% exact confidence interval of 38.36% to 83.71%). The overall negative percent agreement of the VITROS Anti-HBc IgM assay with the reference anti-HBc IgM assay in Population I was estimated to be 99.76% (1668/1672, with a 95% exact confidence interval of 99.39% to 99.93%). As a result of this study, in Population II the overall positive percent agreement of the VITROS Anti-HBc IgM assay with the reference anti-HBc IgM assay was estimated to be 78.65% (70/89, with a 95% exact confidence interval of 68.69% to 86.63%). The overall negative percent agreement was estimated to be 100.0% (226/226, with a 95% exact confidence interval of 98.38% to 100.0%).

The 26 presumably false negative VITROS anti-HBc IgM results (VITROS anti-HBc IgM negative and reference anti-HBc IgM assay reactive) observed in Populations I and II combined, were tested with a second FDA approved anti-HBc IgM assay at the completion of this study. With 14 of the 26 samples, this second assay gave 14 negative or indeterminate results. Of the four presumably false positive VITROS anti-HBc IgM results observed in Population I, all four were also positive with the second FDA approved assay.

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 IgM assay. All 100 samples were negative.

With samples from individuals with documented acute HBV infection, the VITROS anti-HBc IgM assay was reactive with 8 of 8 samples (100%). When samples from individuals with documented chronic HBV infection were tested, the VITROS Anti-HBc IgM was reactive with 11 of 76 samples (14.5%).

The VITROS anti-HBc IgM assay detected the presence of anti-HBc IgM at the same bleed date as the reference anti-HBc IgM assay in five

of seven commercial seroconversion panels. With two panels, the reference anti-HBc IgM assay detected anti-HBc IgM one bleed earlier (equivalent to four days or less and two days or less, respectively) than the VITROS Anti-HBc IgM assay.

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

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