Laboratory Procedure Manual

Analyte: Hepatitis A Antibody

Matrix: Serum

Method: HAV T – Anti-HAV Total
VITROS Immunodiagnostic Products (REF 680 1823)

Method No.:

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As performed by: *Assay Development and Diagnostic Reference Laboratory*
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Important Information for Users

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

Public Release Data Set Information

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

Data File Name	Variable Name	SAS Label
HEPA_F	LBXHA	Hepatitis A antibody (anti-HAV)

Intended for Use in the United States

INSTRUCTIONS FOR USE HAV T

VITROS Immunodiagnostic Products

Anti-HAV Total Reagent Pack

REF 680 1823

Version 1.1 Pub. No. GEM1235A_EN_US

Intended Use

For the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis A virus (total anti-HAV) in human adult and pediatric serum and plasma (EDTA, heparin or citrate) using the VITROS ECi/ECiQ Immunodiagnostic System.
The assay is indicated, in conjunction with other serological and clinical information, as an aid in the clinical laboratory diagnosis of individuals with acute or past hepatitis A virus infection, or as an aid in the identification of HAV-susceptible individuals prior to HAV vaccination. The detection of HAV-specific antibodies in human serum or plasma is laboratory evidence of acute or recent HAV infection.

WARNING: ***This assay is not intended for screening blood or solid or soft tissue donors. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations.***

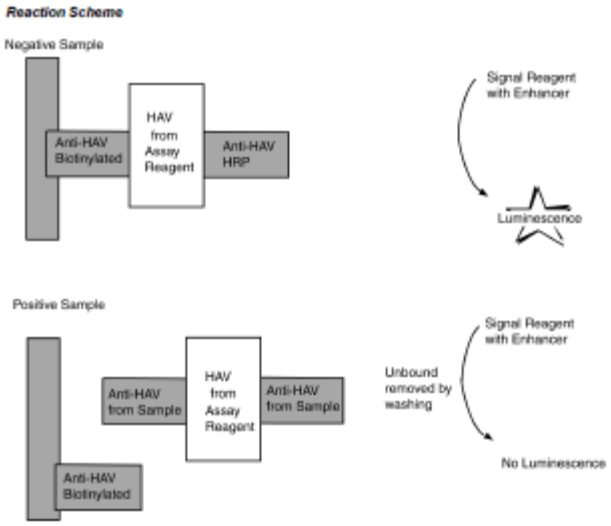
Summary and Explanation of the Test

Hepatitis A virus (HAV) infection is a cause of morbidity and socio-economic loss in many parts of the world.[1,2] Transmission is typically via the fecal-oral route associated with contaminated water or food[1,2,3]. In areas where sanitation is poor, infections often occur early in life. In childhood, HAV infection is generally mild or asymptomatic and results in lifelong immunity. With improved sanitation and hygiene, infections are delayed and consequently the number of adolescents and adults susceptible to the virus increases. In adolescents and adults, HAV infection is more serious leading to hepatitis and an increased mortality rate.[4]
Anti-HAV IgM is detectable during the acute stage of illness, while anti-HAV IgG may be present for many years after recovery[1,2,4] or following vaccination. The presence of anti-HAV (IgG or IgM) in human serum or plasma is indicative of past or present infection with hepatitis A virus (HAV) or vaccination against HAV. The test for total anti-HAV is primarily used to determine exposure to HAV either naturally or due to vaccination.

Principles of the Procedure

The VITROS Anti-HAV Total assay is performed using the VITROS Immunodiagnostic Products Anti-HAV Total Reagent Pack and the VITROS Immunodiagnostic Products Anti-HAV Total Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System (VITROS Immunodiagnostic System).
A competitive immunoassay technique is used which involves pre-incubation of anti-HAV in the sample with HAV antigen in the assay reagent, followed by incubation with a conjugate reagent that contains biotinylated mouse monoclonal anti-HAV antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HAV antibody. The immune complex is captured by streptavidin on the wells. Unbound materials are removed by washing.
The bound HRP conjugate is measured by a luminescent reaction.[5] 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 (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the VITROS Immunodiagnostic System. The binding of HRP is indicative of the absence of anti-HAV antibody.

Assay Type	Assay Time and Temperature	
Competitive	Incubation time:	45 minutes
	Time to first result:	53 minutes
	Temperature:	37° C



Warnings and Precautions

For *in vitro* diagnostic use only.

WARNING: Potentially Infectious Material

The* VITROS *Anti-HAV Total Assay Reagent contains formalin inactivated HAV virus. Treat as potentially infectious.

The* VITROS *Anti-HAV Total Calibrator contains human anti-HAV positive and anti-HAV negative plasma that have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen(HBsAg), and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays, EIA). Treat as if capable of transmitting infection.
Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handling of samples and assay components, their use, storage, and solid and liquid waste disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation (e.g. CLSI Guideline M29).[6,7]

WARNING: Contains Kathon and Proclin 300

The assay reagent and conjugate reagent contain Proclin 300. R43: May cause sensitization by skin contact. R36/38: Irritating to eyes and skin. S23: Do not breathe vapors or spray. S24/25: Avoid contact with skin and eyes.
The calibrator contains Kathon. R43: May cause sensitization by skin contact. R52/R53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S24: Avoid contact with skin. S37: Wear suitable gloves.

Reagents

Reagent Pack Contents

One VITROS Anti-HAV Total Reagent Pack, 100 tests (CAT No. 680 1823) contains:
· 100 coated wells (streptavidin source, bacterial: binding capacity ≥3 ng biotin/well).
· 8.7 mL assay reagent (inactivated HAV antigen [pHM175] source, cell culture; 2-20 mg/mL) in buffer with mouse serum and antimicrobial agent.
· 12.0 mL conjugate reagent (HRP-mouse monoclonal anti-HAV [21D4] 1.5 µg/mL) and biotin-mouse monoclonal anti-HAV 1.5 µg/mL) in buffer with antimicrobial agent.

NOTE: Contains bovine serum albumin.

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-HAV Total 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 12 weeks.
- Store opened reagent packs in the VITROS 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, heparin or citrated plasma.
The differences between serum and citrate samples may be larger than 10% due to the liquid anticoagulant in the tube. There is approximately a 10% dilution of the blood by the liquid anticoagulant in the citrate tubes. (Refer to Matrix Comparison.)

Specimens Not Recommended

It is recommended that turbid samples not be tested.
Do not use heat-inactivated samples.

Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies.[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-HAV Total assay uses 10 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.

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 Clinical and Laboratory Standards Institute (CLSI) [formerly 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) for up to 5 days.

· If the assay will not be completed within 5 days, 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-HAV Total assay:
· VITROS Immunodiagnostic System
· VITROS Anti-HAV Total Calibrator
· VITROS Immunodiagnostic Products Signal Reagent
· VITROS Immunodiagnostic Products Universal Wash Reagent
· Quality control materials, such as VITROS Immunodiagnostic Products Anti-HAV Total 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 Immunodiagnostic System.

Calibration

Sample Dilution

Rare patient samples occur that give high result ratios beyond the normal negative population, and which may be negative or positive for anti-HAV. The results of these samples are flagged "Retest?" and may be resolved by manually diluting the sample 1 in 20 with High Sample Diluent B and retesting. Refer to the High Sample Diluent B instructions for use.

Calibration

Required Calibrator

VITROS Anti-HAV Total Calibrator

Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of the VITROS Anti-HAV Total 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 the calibration process.

When to Calibrate

· Calibrate when the lot of reagent pack and calibrator changes.
· Calibrate every 28 days.
The VITROS Anti-HAV Total assay may also need to be recalibrated:
· After specified service procedures have been performed (refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide).
· If quality control results are 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-HAV 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 (refer to 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 recommended to repeat some or all patient samples, processed after the last acceptable QC results.

· 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.[11] VITROS Anti-HAV Total Controls are recommended for use with the VITROS 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-HAV Total assay.

Quality Control Material Preparation and Storage

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

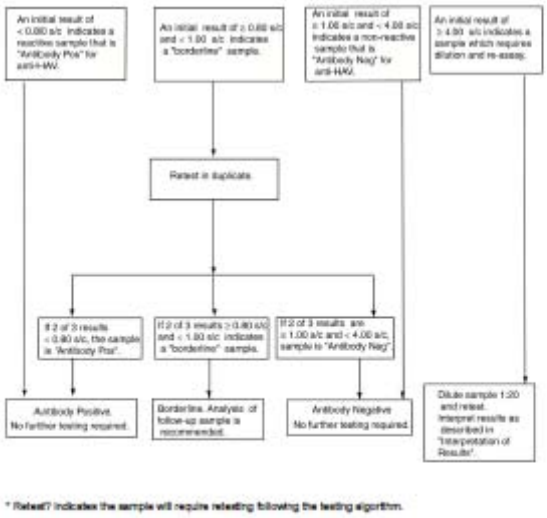
Interpretation of Results and Expected Results

Results are calculated as a normalized signal, relative to a cut-off value (signal/cutoff, s/c). During the calibration process a lotspecific
parameter, encoded on the lot calibration card, is used to determine a valid stored cut-off value for the VITROS Immunodiagnostic System.
Result = Signal for test sample
Cut-off value
Patient sample results will be displayed as "Antibody Pos", "Borderline", "Antibody Neg", or "Retest?"*. An initial result labeled with "Borderline" indicates a sample that requires duplicate repeat testing for anti-HAV. An initial result labeled "Retest?" indicates a sample which requires dilution and re-assay.

Result s/c	< 0.80	≥ 0.80 and < 1.00	≥ 1.00 and < 4.00	≥ 4.00
Result Text	**Antibody Pos**	**Borderline**	**Antibody Neg**	**Retest?***

Final results should be interpreted using the algorithm below.

Testing Algorithm



* Retest? indicates the sample will require retesting following the testing algorithm.

Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS Anti-HAV Total assay upon completion of all testing steps required in the testing algorithm.

VITROS Anti-HAV Total Assay Result	Result Text	Clinical Interpretation
< 0.80	Antibody Pos	Indicates a reactive sample and the presence of anti-HAV. Indicates individual has been previously infected with or is presumed to be immune to HAV infection.
≥ 0.80 and < 1.00	Borderline	Indicates a borderline sample. It is recommended that a new specimen be collected within 2-4 weeks and retested.
≥ 1.00 and < 4.00	Antibody Neg	Indicates a non-reactive sample, negative for anti-HAV. Indicates that the individual has not been infected and is presumed not to be immune to HAV infection.
≥ 4.00	Retest?	Indicates a sample which requires dilution and retesting.

Expected Results

HAV Prevalence Population

The expected results of the VITROS Immunodiagnostic Products Anti-HAV Total assay to detect anti-HAV IgG and IgM were determined in presumably healthy individuals from areas of both high (Western US) and low (Eastern US) HAV disease prevalence in the United States. The population was 50% male and 50% female, with ages that ranged from 18 to 89 years. The majority of the subjects were White/Caucasian (72.0%). Other ethnic groups tested were African American (12.0%), Hispanic/Latino (15.0%) and Asian (1.0%). The expected results for presumably healthy individuals living in either high or low prevalence areas are presented in the table below.

Expected Results for the VITROS Anti-HAV Total Assay in Subjects From Low Prevalence Areas for Hepatitis A (N=648)

Age Range	Gender	VITROS Anti-HAV Total Result						Total
		Reactive		Borderline		Negative		
		N	Percent	N	Percent	N	Percent	
18-20	Female	0	0	0	0	11	100	11
	Male	0	0	0	0	6	100	6
21-30	Female	5	18.5	0	0	22	81.5	27
	Male	6	15.4	0	0	33	84.6	39
31-40	Female	5	19.2	0	0	21	80.8	26
	Male	5	11.1	1	2.2	39	86.7	45
41-50	Female	25	33.3	1	1.3	49	65.3	75
	Male	12	21.8	0	0	43	78.2	55
51-60	Female	21	20.6	0	0	81	79.4	102
	Male	29	23.4	0	0	85	74.6	114
61-70	Female	12	54.5	0	0	10	45.5	22
	Male	19	57.6	0	0	14	42.4	33
71-80	Female	22	71	0	0	9	29	31
	Male	17	81	0	0	4	19	21
81-90	Female	6	85.7	0	0	1	14.3	7
	Male	7	87.5	0	0	1	12.5	8
Total		191	30.7	2*	0.3	429*	69	622

*Three subjects had initial results in the Borderline region.

Expected Results for the VITROS Anti-HAV Total Assay in Subjects From High Prevalence Areas for Hepatitis A (N=378)

Age Range	Gender	VITROS Anti-HAV Total Result						Total
		Reactive		Borderline		Negative		
		N	Percent	N	Percent	N	Percent	
16-20	Female	3	100.0	0	0.0	0	0.0	3
	Male	0	0.0	0	0.0	6	100.0	6
21-30	Female	23	57.5	1	2.5	16	40.0	40
	Male	17	43.6	0	0.0	22	56.4	39
31-40	Female	21	61.8	2	5.9	11	32.4	34
	Male	14	35.9	0	0.0	25	64.1	39
41-50	Female	9	50.0	0	0.0	9	50.0	18
	Male	11	45.8	0	0.0	13	54.2	24
51-60	Female	25	34.7	0	0.0	47	65.3	72
	Male	13	34.2	0	0.0	25	65.8	38
61-70	Female	8	40.0	0	0.0	12	60.0	20
	Male	6	40.0	0	0.0	9	60.0	15
71-80	Female	2	28.6	0	0.0	5	71.4	7
	Male	6	46.2	0	0.0	7	53.8	13
81-90	Female	5	100.0	0	0.0	0	0.0	5
	Male	3	60.0	0	0.0	2	40.0	5
Total		166	43.9	3*	0.8	209	55.3	378

*Three subjects had initial results in the Borderline region.

Adult Subjects at High Risk for Hepatitis

Expected results from asymptomatic individuals from the multi-center study described in “Performance Characteristics” are provided below. Approximately 74.3% (648) of the 872 prospective subjects enrolled in the US reported no recent or current signs or symptoms of hepatitis. Of these 648 asymptomatic individuals, 8.0% were enrolled in Miami, FL, 46.3% were enrolled in Dallas, TX, and 45.7% were enrolled in Chicago, IL. The group was Caucasian (25.6%), African American (55.0%) Hispanic (15.0%), and Asian (1.1%) with the remaining 3.3% represented by other ethnic groups. The group was 58.5% male and 41.5% female and ranged in age from 16 to 81 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS Anti-HAV Total assay was reactive in 50.2% of the individuals in this group. The percent VITROS Anti-HAV Total reactive results observed in the asymptomatic population at each collection site was 4.2% at Miami, FL, 28.2% at Chicago, IL, and 17.8% at Dallas, TX. The expected results for the VITROS Anti-HAV Total assay in subjects at high risk for viral hepatitis are presented in the following table. None of the samples in this group yielded Borderline results

Expected Results for the VITROS Anti-HAV Total Assay in Subjects at High Risk for Viral Hepatitis Without Signs or Symptoms of Hepatitis (N=648)

Age Range	Gender	VITROS Anti-HAV Total Result						Total
		Reactive		Borderline		Negative		
		N	Percent	N	Percent	N	Percent	
16-20	Female	5	45.5	0	0.0	6	54.5	11
	Male	2	28.6	0	0.0	5	71.4	7
21-30	Female	21	35.0	0	0.0	39	65.0	60
	Male	14	26.4	0	0.0	39	73.6	53
31-40	Female	32	40.5	0	0.0	47	59.5	79
	Male	54	39.1	0	0.0	84	60.9	138
41-50	Female	38	64.4	0	0.0	21	35.6	59
	Male	65	55.6	0	0.0	52	44.4	117
51-60	Female	26	74.3	0	0.0	9	25.7	35
	Male	21	61.8	0	0.0	13	38.2	34
61-70	Female	14	73.7	0	0.0	5	26.3	19
	Male	22	91.7	0	0.0	2	8.3	24
71-80	Female	5	100	0	0.0	0	0	5
	Male	5	83.3	0	0.0	1	16.7	6
81-90	Female	1	100	0	0.0	0	0	1
Total		325	50.2	0	0.0	323	49.8	648

There were no initial Borderline results observed in these subjects.

Pediatric Subjects at Low Risk for Hepatitis

Expected results for the VITROS Anti-HAV Total assay were also determined using unlinked, randomly collected samples from pediatric subjects at low risk for viral hepatitis (N=109). The group was 31.2% male and 68.8% female, and the subjects’ ages ranged from 2 to 19 years. The expected results for the VITROS Anti-HAV Total assay in pediatric subjects are presented in the following table.

Expected Results for the VITROS Anti-HAV Total Assay in Pediatric Subjects At Low Risk for Viral Hepatitis (N=109)								
Age Range	Gender	VITROS Anti-HAV Total Result						Total
		Reactive		Borderline		Negative		
		N	Percent	N	Percent	N	Percent	
2-4	Female	0	0.0	0	0.0	6	100	6
	Male	2	16.7	0	0.0	10	83.3	12
5-9	Female	2	8.7	0	0.0	21	91.3	23
	Male	2	22.2	0	0.0	7	77.8	9
10-14	Female	6	22.2	0	0.0	21	77.8	27
	Male	1	20.0	1	20.0	3	60.0	5
15-19	Female	3	15.8	0	0.0	16	84.2	19
	Male	0	0.0	0	0.0	8	100	8
Total		16	14.7	1	0.9	92	84.4	109

Sixteen of 109 specimens were reactive and one had a borderline result with the VITROS Anti-HAV Total assay. The remaining 92 specimens were negative with both the VITROS and reference assays.

Limitations of the Procedure

· This device is more sensitive for anti-IgG than IgM.

· 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 A virus. Levels of anti-HAV antibody may be below the cut-off in early infection and many years after infection. It has been shown that a viremic window exists with individuals infected with HAV where the individual may be symptomatic for hepatitis, but anti-HAV total and anti-HAV IgM nonreactive.

· A reactive anti-HAV total result does not necessarily rule out other hepatitis infections.

· Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays.[12] 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.

· Cord blood and neonate samples may give a negative bias in the VITROS Anti-HAV Total assay. (See Recommended Specimen Types.)

· The magnitude of a VITROS Anti-HAV Total assay result cannot be correlated to an endpoint titer.

· Some anticoagulants (e.g. liquid citrate) have a dilutional effect on samples and results should be interpreted accordingly. (Refer to Matrix Comparison.)

Performance Characteristics

Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS Anti-HAV Total 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 evaluated from 872 subjects prospectively enrolled at three geographically separated collection sites within the United States (Population 1) located in Miami, FL (12.6%), Dallas, TX (37.5%) and Chicago, IL (49.9%). Specimens were also evaluated from 313 subjects prospectively enrolled in an area in India with a high prevalence of viral hepatitis (Population 2). Statistical testing performed to evaluate the homogeneity of the distribution of VITROS Anti-HAV Total assay s/c values across the four collection sites indicated that the data from Population 1 and Population 2 could be pooled for statistical analysis.
The subjects in Population 1 were Caucasian (25.6%), African American (53.1%), Hispanic (17.0%), with the remaining 4.3% represented by other ethnic groups. The group was 57.3% male and 42.7% female, and ranged in age from 16 to 81 years. Testing of these specimens with the VITROS Anti-HAV Total assay occurred at diagnostic laboratories located in Miami, FL (12.6%), Port Jefferson, NY (49.9%) and Minneapolis, MN (37.5%).
The subjects in Population 2 were Indian (100.0%). The group was 72.8% male and 27.2% female, and ranged in age from 18 to 90 years. Testing of these specimens with the VITROS Anti-HAV Total assay occurred at diagnostic laboratories located in Miami, FL (43.8%), Minneapolis, MN (43.8%) and Port Jefferson, NY (12.5%).

Agreement of the VITROS Anti-HAV Total assay was assessed relative to the reference anti-HAV total assay using serum samples from Population 1, Population 2, and Populations 1 and 2 combined.

Percent Agreement

A comparison of the VITROS Anti-HAV Total assay and the reference anti-HAV Total assay results is presented in the following tables. Data are listed by site and population. Positive and negative percent agreement and 95% exact confidence intervals are also shown.

VITROS and Reference Anti-HAV Total Assay Results in Population 1: Prospective Samples from the U.S. (N=872)								
VITROS Anti-HAV Total Assay Result	Reference Anti-HAV Total Assay Result							
	Site 1		Site 2		Site 3		All Sites	
	Reactive	Negative	Reactive	Negative	Reactive	Negative	Reactive	Negative
Reactive	61	2	287	3	121	4	449	9
Borderline	0	0	0	0	0	0	0	0
Negative	0	47	1	164	1	201	2	412
Total	61	49	288	167	122	205	451	421
Positive Percent Agreement	100% (61/61)		99.83% (287/288)		99.18% (121/122)		99.56% (449/451)	
95% Exact Confidence Interval	94.13% - 100%		97.94% - 99.99%		95.52% - 99.98%		98.41% - 99.95%	
Negative Percent Agreement	95.92% (47/49)		98.20% (164/167)		98.05% (201/205)		97.86% (412/421)	
95% Exact Confidence Interval	86.02% - 99.50%		94.84% - 99.63%		95.08% - 99.47%		95.98% - 99.02%	

There were no initial borderline results observed in these populations.

VITROS and Reference Anti-HAV Total Assay Results in Population 2: Prospective Samples from India (N=313)								
VITROS Anti-HAV Total Assay Result	Reference Anti-HAV Total Assay Result							
	Site 1		Site 2		Site 3		All Sites	
	Reactive	Negative	Reactive	Negative	Reactive	Negative	Reactive	Negative
Reactive	135	2	39	0	133	4	307	6
Borderline	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0
Total	135	2	39	0	133	4	307	6
Positive Percent Agreement	100% (135/135)		100% (39/39)		100% (133/133)		100% (307/307)	
95% Exact Confidence Interval	97.30% - 100%		90.97% - 100%		97.26% - 100%		98.81% - 100%	
Negative Percent Agreement	0% (0/2)		N/A		0% (0/4)		0% (0/6)	
95% Exact Confidence Interval	0% - 84.19%		N/A		0% - 60.24%		0% - 45.93%	

There were no initial borderline results observed in these populations.

The positive percent agreement of the VITROS Anti-HAV Total assay with the reference anti-HAV total assay was 99.56% (449/451) for Population 1 and 100% (307/307) for Population 2. The negative percent agreement of the VITROS Anti-HAV Total assay with the reference assay was 97.86% (412/421) for Population 1 and 0% (0/6) for Population 2.
The overall positive percent agreement for the VITROS Anti-HAV Total assay with the reference assay was 99.74% (756/758), with a 95% exact confidence interval of 99.05% to 99.97% for the prospective samples in Populations 1 and 2 combined. The overall negative percent agreement for the VITROS Anti-HAV Total assay with the reference assay was 96.49% (412/427), with a 95% exact confidence interval of 94.27% to 98.02% for the prospective samples in Populations 1 and 2 combined.

Performance of the VITROS Anti-HAV Total Assay in Known Anti-HAV IgM Reactive Subjects

The performance of the VITROS Anti-HAV Total assay was evaluated among serum samples from subjects known to be anti-HAV IgM positive.
A total of 77 samples collected in Egypt (N=50) and India (N=27) from subjects with a medical history and laboratory results indicative of acute hepatitis A were tested concurrently with the VITROS and reference anti-HAV IgM and anti-HAV total assays.
The VITROS Anti-HAV Total assay was reactive in 100% of the 77 anti-HAV IgM reactive samples. The percent agreement of the VITROS Anti-HAV Total assay with the reference assay and the 95% exact confidence interval are 96.1% (74/77) and 89.0% - 99.2% respectively.
The reference anti-HAV total assay was negative in three subjects. The three reactive results with VITROS Anti-HAV Total assay was considered falsely reactive for purposes of the analysis

Performance of the VITROS Anti-HAV Total Assay in Pediatric Subjects

The VITROS Anti-HAV Total assay was also evaluated using residual laboratory serum samples from pediatric subjects at low risk for viral hepatitis. The samples were unlinked to the subjects' identities, and were included based on age, gender and available volume remaining after all testing ordered for that sample had been completed. Samples were selected such that the following age ranges (in years) were represented (2-4, 5-9, 10-14, and 15-19).
The positive and negative percent agreement of the VITROS Anti-HAV Total assay with the reference anti-HAV total assay, and the 95% exact confidence intervals are presented in the following table. One sample, negative with the reference assay,

was Borderline with the VITROS Anti-HAV Total assay and was considered falsely reactive for purposes of the analysis.

Agreement of the VITROS and Reference Anti-HAV Total Assays in Pediatric Subjects				
Population	Positive Percent Agreement	95% Exact Confidence Intervals	Negative Percent Agreement	95% Exact Confidence Intervals
Pediatric Subjects	93.75% (15/16)	69.77% - 99.84%	97.85% (91/93)	92.45% - 99.74%

The positive percent agreement for the VITROS Anti-HAV Total assay with the reference assay was 93.75% (15/16), with a 95% exact confidence interval of 69.77% to 99.84% for the pediatric samples. The negative percent agreement for the VITROS Anti-HAV Total assay with the reference assay was 97.85% (91/93), with a 95% exact confidence interval of 92.45% to 99.74% for the pediatric samples.

Performance of the VITROS Anti-HAV Total Assay in Cord Blood

A total of 20 cord blood (as a surrogate for neonate serum) and 10 adult serum samples were tested in the VITROS Anti-HAV Total assay. None of the samples gave a reactive result in the VITROS Anti-HAV Total assay. Forty-five (45) μl of anti-HAV positive material was added to 255 μl of cord blood and adult serum. A negative bias* was observed in the cord blood results when compared to the adult serum.

Anti-HAV Total Cord Blood Study			
Sample Type	N	Mean Response (S/C)	St Dev
Cord Blood - Neat	20	2.17	0.205
Cord Blood - Spiked	20	0.89	0.151
Adult Serum - Neat	10	2.19	0.075
Adult Serum- Spiked	10	0.77	0.022

*Due to the competitive nature of the VITROS Anti-HAV Total assay a positive numerical bias indicates a negative functional bias.

Seroconversion Panels

Three seroconversion panels each having at least 5 individual samples with a known predetermined result were measured in the VITROS Anti-HAV Total assay and in a reference assay. The results were compared with the published results for the reference assay. The VITROS Anti-HAV Total assay gave seroconversion sensitivity equivalent to or more sensitive than a reference assay in the three panels tested.

Panel ID	VITROS Anti-HAV Total		Anti-HAV Total Reference Assay		Difference in Days to Reactive Result
	Post bleed day of last non-reactive result	Post bleed day of first reactive result	Post bleed day of last non-reactive result	Post bleed day of first reactive result	
PHT902	3	16	3	16	0
RP-013	6	9	6	9	0
RP-004	P*	1	1	7	6

P* = Positive in first bleed

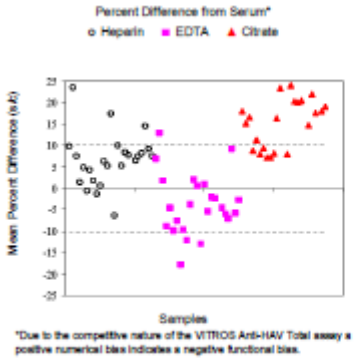
Potentially Cross-Reacting Subgroups

The specificity of the VITROS Anti-HAV Total assay was evaluated by testing 283 samples from the following potentially crossreacting
sub-groups: SLE, anti-HIV, Cirrhosis, Non-viral Liver Disease, anti-HCV, anti-CMV, anti-HSV I & II, anti-EBV, antisyphilis, anti-Rubella, anti-Toxoplasma, anti-HBs, anti-HTLV, HBsAg, Rheumatoid Factor, Pregnancy (1st – 3rd Trimester), HAMA, Rubeola, Mumps, VZV and ANA. All initially reactive samples were tested with a reference assay for confirmation. None of these categories were found to interfere with the VITROS Anti-HAV Total assay.
Of the 283 samples tested, four (4) were observed to be discordant. The incidence of discordant samples is not significantly different from the claimed sensitivity and specificity.

Summary of Data from Potentially Cross-Reacting Sub-Groups					
Sample Category	No. Samples Tested	VITROS Anti-HAV Total Assay		Reference Assay	
		No. Negatives	No. Initial Reactives/Borderlines	No. Initial Reactives/Borderlines	No. Discordants
Toxo IgM	4	3	1	1	0
Toxo IgG	20	6	14	13	1
RF	50	24	26	25	1
SLE	10	5	5	5	0
HIV	10	7	3	2	1
aHBs	10	2	8	8	0
HCV	10	7	3	3	0
Cirrhosis	10	7	3	3	0
Syphilis	10	9	1	1	0
HTLV	10	8	2	2	0
Non Viral Liver	12	4	8	8	0
HBsAg	10	4	6	6	0
HAMA	9	5	4	4	0
EBV	10	9	1	0	1
1st Trimester	18	10	8	8	0
2nd Trimester	16	7	9	9	0
3rd Trimester	16	7	9	9	0
HSV 1	3	3	0	N/A	N/A
HSV 2	2	2	0	0	0
CMV	8	8	0	N/A	N/A
VZV	5	5	0	0	0
Mumps	5	5	0	0	0
Rubella	15	12	3	3	0
Rubeola	5	3	2	2	0
ANA	5	3	2	2	0
Total	283	165	118	114	4

Matrix Comparison

A total of 25 donors had blood drawn which was spiked with anti-HAV total positive plasma to a level close to the assay cut-off. The spiked blood was then aliquoted into serum and plasma collection tubes and tested in the VITROS Anti-HAV Total assay. The percent difference in the plasma from serum was calculated. Mean percent differences from serum are represented below for each plasma type tested.



*Due to the competitive nature of the VITROS Anti-HAV Total assay a positive numerical bias indicates a negative functional bias.

Some anti-coagulants (e.g. liquid citrate) have a dilutional effect on samples and results should be interpreted accordingly.

Substances that do not Interfere

Serial dilutions were made for bilirubin, triolein, hemoglobin and biotin, and point estimates were made for sodium azide and dipyrone. The mean result of 3 determinations of a solution of each test substance was compared with that of a control pool, for both a negative and positive sample. For each substance, the highest concentration which was considered not to impact results, the mean result from the three kit lots and the classification for both positive and negative samples are shown in the table below.

Compound	Compound Concentration	
Bilirubin	0.342 mmol/L	20 mg/dL
Biotin	10 ng/mL	1 µg/dL
Dipyrone	1 mg/mL	10 mg/dL
Hemoglobin	0.078mmol/L	125 mg/dL
Sodium Azide	1 g/dL	1000 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

Precision

Precision was evaluated according to the Clinical and Laboratory Standards Institute (formerly NCCLS) protocol EP5-A2[13]. The precision panel consisting of 4 samples (a negative, a negative close to the cut-off, a positive close to the cut-off and a positive)

was prepared and shipped to 3 different sites. Two replicates of each of 4 panel samples were assayed at each of the 3 different sites once per day for at least 20 different days, over one calibration interval. The experiment was performed using 1 reagent lot on three different VITROS Immunodiagnostic Systems at three different sites. The data presented is a summary of the product performance.

Clinical Site	Mean VITROS Anti-HAV Total S/C (Ratio)	Within Day*		Between Day**		Total***		No. of Observ.	No. of Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Site 1	1.74	0.015	0.9	0.041	2.3	0.043	2.5	40	20
	1.08	0.018	1.6	0.026	2.4	0.031	2.9	40	20
	0.73	0.008	1.1	0.019	2.6	0.020	2.8	40	20
	0.52	0.006	1.1	0.012	2.3	0.013	2.6	40	20
Site 2	1.93	0.054	2.8	0.029	1.5	0.061	3.2	40	20
	1.14	0.015	1.3	0.033	2.9	0.036	3.2	40	20
	0.74	0.011	1.5	0.028	3.8	0.030	4.0	40	20
	0.51	0.007	1.3	0.026	5.1	0.027	5.3	40	20
Site 3	1.93	0.029	1.5	0.068	3.5	0.074	3.8	40	20
	1.18	0.030	2.5	0.038	3.2	0.048	4.1	40	20
	0.77	0.013	1.8	0.030	3.8	0.032	4.2	40	20
	0.55	0.012	2.2	0.030	5.4	0.032	5.8	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

Mean VITROS Anti-HAV Total S/C (Ratio)	Between Site *		Total **		No. Obs.
	SD	CV (%)	SD	CV (%)	
1.87	0.108	5.8	0.124	6.7	120
1.13	0.048	4.2	0.062	5.5	120
0.75	0.023	3.1	0.036	4.9	120
0.52	0.020	3.7	0.032	6.1	120

* Between Site: Variability of the assay performance from site to site.
** Total: Variability of the assay incorporating factors of site and day.

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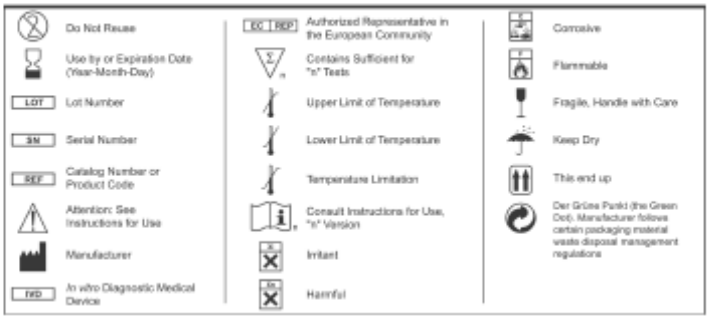
Glossary of Symbols

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



Revision History

Date of Revision	Version	Description of Technical Changes*
2007-10-04	1.1	Address Block: CHIRON Corporation changed to Novartis Vaccines and Diagnostics, Inc.
2006-09-20	1.0	Initial version of Instructions for Use.

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

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

Signature _____ Obsolete Date _____

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