Laboratory Procedure Manual

Analyte: **Hepatitis A Antibody**

Matrix: **Serum**

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

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As performed by: **Diagnostic Reference Team**
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Important Information for Users

The National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention 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_K	LBXHA	Hepatitis A antibody (anti-HAV)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical relevance:

Hepatitis is inflammation of the liver most often caused by a virus. Viral hepatitis is a major public health problem of global importance because of the ongoing transmission of viruses that cause the disease, increased morbidity and mortality associated with the acute and chronic consequences of these infections. Global and US goals have been established for elimination of viral hepatitis as a public health threat by 2030.

In the US, the most common types of viral hepatitis are hepatitis A, B, and C. Effective vaccines are available to help prevent hepatitis A and hepatitis B. No vaccine is available for hepatitis C; however, highly effective, well-tolerated treatment can cure hepatitis C virus infection. Hepatitis D virus infection is less common in the US and can occur only among persons with hepatitis B virus infection. Hepatitis E infection also is less common in the US. These five hepatitis viruses, also called hepatitides, are well-characterized for detection with laboratory assays and are monitored in U.S. public health surveillance systems.

NHANES viral hepatitis data are used to monitor progress toward goals in Healthy People and the HHS Viral Hepatitis National Strategic Plan, which in turn support US and global viral hepatitis elimination goals. The viral hepatitis laboratory and interview components of NHANES complement data from outbreak, case-based surveillance, vital statistics, health care systems, and cohort studies that can provide timely, detailed, or longitudinal information for subnational geographic areas and disproportionately affected populations (such as persons experiencing homelessness or living in correctional facilities), but may lack information available from NHANES, such as race, ethnicity, education, income, and health status and behavior.

Viral hepatitis data from NHANES are available beginning with the Second NHANES conducted during 1976-1980 for hepatitis A and hepatitis B, and with the Third NHANES conducted during 1988-1994 for hepatitis C, hepatitis D and hepatitis E.

Hepatitis A virus (HAV) infection is a cause of morbidity and socio-economic loss in many parts of the world. Transmission is typically via the fecal-oral route associated with contaminated water or food. 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.

Anti-HAV IgM is detectable during the acute stage of illness, while anti-HAV IgG may be present for many years after recovery 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. No test can differentiate between exposure naturally or due to vaccination.

The total anti-HAV test is used for the NHANES viral hepatitis component.

Examined participants aged 2 years and older in the NHANES 2019-March 2020 sample were eligible for the hepatitis A antibody test.

Test principle:

Hepatitis A antibody (anti-HAV) is measured using the VITROS Anti-HAV Total assay. The test is performed using the VITROS Anti-HAV Total Reagent Pack and the VITROS Anti-HAV Total Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System. A competitive immunoassay technique is used, which involves pre-incubation of anti-HAV in the sample with HAV antigen in the test 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. Unbound materials are 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 (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The binding of HRP conjugate is indicative of the absence of anti-HAV antibody.

2. SAFETY PRECAUTIONS:

CIA test kits for anti-HAV contain components derived from human serum or plasma. Although various treatments in the manufacturing process are sufficient to inactivate most blood-borne pathogens, there is no assurance that these reagents are entirely noninfectious. Therefore, treat components of test kits as though they are capable of transmitting disease.

Consider all serum specimens for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of infectious contamination hazards. Place all plastic and glassware contaminated with

serum in a plastic autoclave bag for disposal. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished. Biosafety Level 2 containment and practice as described in CDC/NIH publication #88-8395 are recommended for handling test specimens and kit reagents.

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.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Data Management System (DMS) was used until December 31st, 2019.

The run information can be uploaded into the computerized database - DMS - after the run information is exported by the software to the computerized database- DMS. This database was custom-designed for the management of CDC Division of Viral Hepatitis (DVH) Laboratory Branch (LB) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. In August 2019, laboratory data management was transferred to the CDC Enterprise Laboratory Information System (ELIMS), where NHANES functionality was reproduced and improved over time to include more process automation. DMS was maintained in parallel until December 31st, 2019, when it was discontinued. Finished DMS data were reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. Files stored on the CDC Local Area Network (LAN) were automatically backed up nightly by CDC Data Center staff. Documentation for data system maintenance was maintained with printed copies of data records for 2 years.

CDC Enterprise Laboratory Information System (ELIMS) has been used since January 1st, 2020, for accessioning, test results processing, reporting and storage. Finished ELIMS data are reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. All information about the accessioned specimens, traceability of the diagnostic process, test runs and reported results are stored in the ELIMS database, are archived after 12 months and can be retrieved any time upon request. All necessary information about ELIMS at CDC is maintained on an intranet site accessible by CDC laboratory personnel.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- EDTA plasma
- Heparin plasma
- Citrate plasma

Specimens Not Recommended

- Do not use turbid specimens. Turbidity in specimens may affect test results.
- Do not use heat-inactivated samples.

Specimen Collection and Preparation

- Collect specimens using standard procedures.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.
- The VITROS Anti-HAV Total test uses 10 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the operating instructions for your system.
- Return to 2–8 °C (36–46 °F) as soon as possible after use or load sufficient volume for a single determination.
- Serum and plasma samples may be stored for up to 5 days at 2–8 °C (36–46 °F) or 4 weeks at -20 °C (-4 °F).
- The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for storing specimens:
  - Store samples at 22°C (72°F) for no longer than 8 hours.
  - If the test will not be completed within 8 hours, refrigerate samples at 2°–8°C (36°–46°F) for up to 5 days.
  - If the test 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.
- Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29-A. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation and Software

VITROS ECi/ECiQ Immunodiagnostic Systems
VITROS 3600 Immunodiagnostic System

b. Required Materials Not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- VITROS Immunodiagnostic Products High Sample Diluent B
- Quality control materials such as VITROS Immunodiagnostic Products Anti-HAV Total Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

c. Materials Provided

- VITROS Immunodiagnostic Products Anti-HAV Total Reagent Pack
- VITROS Immunodiagnostic Products Anti-HAV Total Calibrator

d. Reagent Preparation

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (streptavidin, bacterial; binding capacity ≥3 ng biotin/well)
- 8.7 mL assay reagent (inactivated HAV antigen [pHM175], 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

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading onto the system.
- Handle the reagent pack with care. Avoid the following:
  – allowing condensation to form on the pack
  – causing reagents to foam
  – agitation of the pack

Reagent Pack Storage and Preparation

Reagent	Storage Condition	Stability
Unopened	Refrigerated 2-8°C (36–46 °F)	Expiration date
Opened	On system System turned on	< 12 weeks
Opened	Refrigerated 2-8°C (36–46 °F)	< 12 weeks

- The VITROS Anti-HAV Total Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

e. Standards Preparation

This method does not involve the use of conventional calibrators or standards. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and Integrated Systems.

f. Preparation of Quality Control Material

Kit positive and negative controls are prepared, and quality controlled by the manufacturer.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibrator Contents

- 1 vial of VITROS Anti-HAV Total Calibrator (human anti-HAV plasma, 2.0mL) with antimicrobial agent
- Lot calibration card
- Protocol card
- 8 calibrator bar code labels

b. Calibrator Handling

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

Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8°C (36–46°F) as soon as possible after use, or load only sufficient for a single determination.

c. Calibrator Storage and Preparation

Calibrator	Storage Condition	Stability
Unopened	Refrigerated 2–8°C (36–46°F)	expiration date
Opened	Refrigerated 2–8°C (36–46°F)	≤ 13 weeks
Opened	Frozen -20°C (-4°F)	≤ 13 weeks

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) The VITROS Anti-HAV Total Reagent Pack is used for 100 tests. Reagent pack is supplied ready to use and its kit components cannot be

interchanged within a manufacturer's lot or between lots. Opened reagent pack must be used within 12 weeks.

(2) Unopened reagent pack is stored refrigerated at 2-8°C (36–46 °F); do not freeze.

(3) Reagent pack is loaded on the instrument directly from refrigerated storage to minimize condensation.

(4) Prepare run sheets, listing controls and specimens in the order presented in the e-file.

(5) Perform daily maintenance of the VITROS instruments according to user manual; verify the validity of the calibrators and if needed update. Run negative and positive controls.

b. **Sample Preparation**

(1) Bring serum specimens and controls from the refrigerators to the bench, mix each vial by inversion and allow 20-30 minutes to reach ambient temperature (15-30°C [59–86 °F]) before use.

Spin down the specimens at 5000 RPM speed for 5 minutes using a swing-bucket centrifuge (Eppendorf Centrifuge 5804/Rotor A-4-44, or similar).

(2) Identify the reaction tray wells for each specimen or control.

c. **Instrument Setup**

(1) Test procedure is performed as described in VITROS 3600 Operation and Maintenance Procedure and VITROS® Anti-HAV Total (aHAVT) Technical Procedure using the VITROS Anti-HAV Reagent Pack and associated controls and calibrator packs. Do not use expired reagents.

(2) During the use of the Data Management System:

a. Take off and discard screw caps from the cryo-vials and then load them in batches of 10 on the VITROS carousels. Ensure that the specimen ID barcode is readable in the holder’s window.

b. Interface the Data Management System (DMS) with the VITROS instrument and submit the run sheet.

c. Start the run and observe the transfer to make sure that all the specimens on the run sheet were scanned by the instrument before the test begins. If a barcode cannot be scanned due to incorrect positioning or an unreadable label, enter specimen ID manually.

d. After completion of the test, interface DMS with the VITROS instrument and import the results into the DMS.

(3) During the use of the Enterprise Laboratory Information Management System (ELIMS):

a. The specimens arriving at CDC are accessioned by the Specimen Triage and Tracking Team into ELIMS for Unit 90 under CLIA regulated practices, and then Unit 90 is notified.
b. Testing Personnel (TP) within Unit 90 pick-up specimens within 24 hours of notice.
c. DRT TP transports the specimens to DRT Pre-analytic testing facilities. Specimens are securely stored ensuring conditions described in the Office of the Associate Director for Laboratory Science and Safety (OADLSS) Biosafety Manual.
d. Process specimens in ELIMS by following the Pre-Analytic Workflow of the ELIMS Job Aids for User Level 2 Data Manager.
e. Fill out the VITROS Runsheet.
f. Before testing, samples in cryovials will be centrifuged at 5000 g for 5 minutes.
g. Upon completion of centrifugation, cryovials shall be open and all the caps shall be disposed.
h. Place cryovials in the VITROS trays, up to 10 vials at a time.
i. Once the results are generated by the VITROS 3600, download the data into ELIMS according to the Post-Analytic Workflow of the ELIMS Job Aid for User Level 2 Data Manager.
j. Once completed, notify the supervisor.

d. **Operation of Assay Procedure**

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.
For detailed information refer to the operating instructions for your system.

e. Recording of Data

(1) Quality Control Data

Quality control is measured using manufacturer provided positive and negative controls.

VITROS Anti-HAV Total Negative Control should generate Antibody Negative results.

VITROS Anti-HAV Total Positive Control should generate Antibody Positive results.

Only runs that pass with controls within the designated range are reported f rom the analyzer.

Controls that fall outside the acceptable range should result in an immediate retest.

If controls fail and are outside of acceptable range, no specimens are tested on the analyzer until controls fall within range.

Raw data are processed by the VITROS Immunodiagnostics System and transferred automatically from the VITROS instrument into the DMS or ELIMS.

(2) Analytical Results

Results are calculated as a normalized signal, relative to a cutoff value (signal/cutoff, s/c). During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and Integrated Systems.

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-test.

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

f. **Replacement and Periodic Maintenance of Key Components**

(1) Instruments are on service contract and, except for the routine daily, weekly, and monthly maintenance, are serviced by an Ortho Clinical Diagnostics technical representative.

Laboratory personnel monitor and document refrigerator temperature, freezer temperature, and room temperature on daily basis.

(2) All micro-pipettors used in testing clinical specimens are calibrated every 6 months. Pipettors that do not conform to specifications are removed from the workflow, disinfected and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

g. **Calibrations**

Calibration Procedure

- Calibration and maintenance procedures/requirements for each piece of equipment can be found in procedure VITROS 3600 Operation and Maintenance SOP.
- Calibration is Lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration is established for each new reagent lot by performing multiple tests. This is the process by which a lot-specific parameter [a] which links the signal at the cutoff (cutoff value) to the calibrator signal is determined. Cutoff value = (a x Signal of Cal 1).
- Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.
- When the calibrator is processed, the validity of the calibration is assessed against quality parameters which compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cutoff value is calculated and stored for use with any reagent pack of that lot.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.

Calibration Model

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process a lot-specific parameter, encoded on the lot calibration card, is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and VITROS Integrated Systems.

h. **Interpretation of results**

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

VITROS Anti-HAV Total Test Result	Result Text	Clinical Interpretation
<0.80	Antibody Positive	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. Result interpreted as Indeterminate.
≥1.00 and <4.00	Antibody Negative	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.

9. REPORTABLE RANGE OF RESULTS

Final results are expressed qualitatively as positive, negative or Indeterminate for the presence of anti-HAV antibody in the sample. No quantitative results are determined.

Expected Results for different HAV Prevalence Population

The expected results of the VITROS Immunodiagnostic Products Anti-HAV Total test 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.

Expected results for the VITROS anti-HAV total test in subjects from low prevalence areas for HepatitisA (N=648) were: Reactive - 191 (30.7%), Borderline – 2 (0.3%), and Negative – 429 (69%).
Expected results for the VITROS anti-HAV total test in subjects from high prevalence areas for HepatitisA (N=378) were: Reactive - 166 (43.9%), Borderline – 3 (0.8%), and Negative – 209 (55.3%).

10. QUALITY CONTROL (QC) PROCEDURES

Quality Control Material Selection

Quality control is measured using manufacturer provided VITROS Anti-HAV Total Controls positive and negative controls for use with the VITROS Immunodiagnostic and VITROS Integrated Systems.

VITROS Anti-HAV Total Negative Control should generate antibody negative results.

VITROS Anti-HAV Total Positive Control should generate antibody positive results.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Anti-HAV Total test. Only runs that pass with controls within the designated range are reported from the analyzer.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.

Positive Controls

Control	Frequency (every run, every extraction, etc.)	Expected Value
VITROS Anti-HAV Total Positive Control	After calibration; At least once each day that the test is being performed; After specified service procedure or maintenance to critical parts or subsystems that might influence performance of the test.	Antibody positive

Negative Controls

Control	Frequency (every run, every extraction, etc.)	Expected Value
VITROS Anti-HAV Total Negative Control	After calibration; At least once each day that the test is being performed; After specified service procedure or maintenance to critical parts or subsystems that might influence performance of the test.	Antibody negative

Additional Controls

Control	Frequency (every run, every extraction, etc.)	Expected Value
None	Not applicable	Not applicable

Quality Control Procedures

Controls that fall outside the acceptable range should result in an immediate retest.

If a second failure results, refer to VITROS 3600 Operation and Maintenance Manual.

If a third failure results, call Ortho CDC VITROS technical support line to troubleshoot either by phone, or schedule field service visit.

If failure occurs subsequently 3 times, TP submits a Nonconforming event report .

NOTE: No specimens are tested and no results are generated from the analyzer if controls fail and are outside of acceptable range.

Quality Control Material Preparation and Storage

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

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

a. If controls do not conform to specifications, reject the results and reanalyze all samples. Do not use data from non-qualifying test runs.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Known Interferences

The VITROS Anti-HAV Total test was evaluated for interference consistent with CLSI document EP7. Commonly encountered substances were tested on 3 lots of reagents. Of the compounds tested, none was found to interfere with the clinical interpretation of the test.

Refer to “Substances that do not Interfere” for a list of compounds tested that did not show interference.

Other Limitations

- 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 cutoff 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. 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 test.
- The magnitude of a VITROS Anti-HAV Total test 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.
- Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration.

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 for both positive and negative samples is shown in the table below.

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	0.078 mmol/L	125 mg/dL
Sodium Azide	1 g/dL	1000 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

13. REFERENCE RANGES (NORMAL VALUES)

The reference value for Anti-HAV Total is antibody negative in a healthy, unexposed population.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 15-25°C (59–77 °F) during preparation and testing for 4 hours.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other FDA-licensed tests for total anti-HAV may be substituted but must be accompanied by validation data to show substantial equivalence with these assays. Test methods may not be substituted without approval from NCHS.

Alternative methods of storage are not recommended. In case of system failure, samples should be refrigerated at 2-8°C (36–46 °F) for no more than 5 days. For longer periods, the specimens should be stored at - 20°C (-4 °F) or below until the system is functioning properly.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database, as described in Section 3, to track specimens.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data. For NHANES, residual specimens are stored frozen at -20^{o}C or below, and returned to the NCHS specimen bank after testing for each cycle has been completed.

19. Summary Statistics and QC graphs

Qualitative assays are assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. The controls are read as cutoff values. However, QC numeric data are monitored over time using Quality Control Charts to detect trends or shifts in performance through regular, planned reviews by supervisors, i.e., monthly, or quarterly. Reviews are used to identify opportunities for improvements.
Evaluation of the Quality Control of Test Results on VITROS Immunodiagnostic System is conducted according to the internal laboratory SOP document.

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