

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: Assay Development and Diagnostic Reference Laboratory
 Laboratory Branch
 Division of Viral Hepatitis
 National Center for HIV/AIDS, Viral Hepatitis, STD, and TB
 Prevention

Contact: Saleem Kamili, PhD (+1-404-639-4431); sek6@cdc.gov

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_J	LBXHA	Hepatitis A antibody (anti-HAV)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

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.

The VITROS Anti-HAV Total 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

- a. The run information can be uploaded into the computerized database after the run information is exported by the software to the computerized database. This database was custom-designed for the management of CDC Assay Development and Diagnostic Reference Laboratory (ADDRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. Test values are compared with a lot-specific cutoff value. Results are expressed as "positive" or "negative" for anti-HAV. Other information in the database may typically include the ADDRL identification number, the specimen number, the date collected, the date tested, and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer.
- b. Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with the other NHANES IV data.
- c. Files stored on the LAN are automatically backed up nightly to tape by CDC Data Center staff.
- d. Documentation for data system maintenance is contained in hard copies of data records for 2 years.

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 for a single volume 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.

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. 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

b. Materials Provided

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

c. 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 $\mu\text{g/mL}$ and biotin-mouse monoclonal anti-HAV 1.5 $\mu\text{g/mL}$) 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
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Unopened	Refrigerated 2-8°C (36–46 °F)	Expiration date
Opened	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.

d. **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.

e. **Preparation of Quality Control Material**

- (1) Kit positive and negative controls are prepared and quality controlled by the manufacturer.
- (2) In-house controls are prepared according to ADDRL specifications.

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; 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) 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) 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.
- (2) Interface the Data Management System (DMS) with the VITROS instrument and submit the run sheet.
- (3) 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.
- (4) After completion of the test, interface DMS with the VITROS instrument and import the results into the DMS.

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

Multiple positive and negative controls are averaged by the VITROS Immunodiagnosics Systems and are determined to be valid or invalid. Quality control of individual control values is maintained by the VITROS Immunodiagnosics System, which will not proceed to the test run if control values do not conform to specifications. Only valid controls data will allow running the test and only valid matching control data on the DMS will allow import of the test results into the DMS.

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

(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-assay.

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 a daily basis.

- (2) All micropipettors used in testing clinical specimens are calibrated every 6 months. Pipettors that do not conform to specifications are autoclaved 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 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).

- Ensure that the Master calibration for each new reagent lot is available on your system.
- 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.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration, refer to the operating instructions for your system.

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 of the VITROS Anti-HAV Total test is traceable to an in-house reference calibrator which has been value assigned to optimize the clinical sensitivity and specificity performance.

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. It is recommended that a new specimen be collected within 2–4 weeks and retested.
≥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 or negative for the presence of anti-HAV antibody in the sample. No quantitative results are determined.

10. QUALITY CONTROL (QC) PROCEDURES

Quality Control Material Selection

VITROS Anti-HAV Total Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. There are 2 VITROS Anti-HAV Total Controls (anti-HAV negative and anti-HAV positive). The performance of other commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other anti-HAV methods if they contain high concentrations of preservatives, stabilizers, or other non-physiological additives, or otherwise depart from a true human sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Anti-HAV Total test.

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- 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
 - According to local regulations or at least once each day that the test is being performed
 - After specified service procedures are performed or maintenance to critical parts or subsystems that might influence performance of the testIf quality control procedures within your laboratory require more frequent use of controls, follow those procedures.
- 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.
- Refer to the published guidelines for general quality control recommendations.
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
For more detailed information, refer to the operating instructions for your system.

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. (See Recommended Specimen Types.)
- 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. Refer to Matrix Comparison.)
- 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.

13. REFERENCE RANGES (NORMAL VALUES)

A normal human serum should be negative for hepatitis A antibodies.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25°C 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 4-8°C for no more than 5 days. For longer periods, the specimens should be stored at - 20°C 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 (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 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. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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