Laboratory Procedure Manual

Analyte: **Hepatitis C Antibody / Hepatitis C Confirmatory Test (Anti-HCV)**

Matrix: **Serum**

Method: **aHCV – Anti-HCV**
VITROS Immunodiagnostic Products (REF 680 1325 and Fujirebio INNO-LIA HCV Score Assay)

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As performed by: **Diagnostic Reference Team Laboratory**
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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:

Dataset Name	Variable Name	SAS Label
HEPC_L	LBDHCI	Hepatitis C antibody (confirmed)

ANTI-HCV SCREENING TEST – VITROS Immunodiagnostic Products aHCV – Anti-HCV

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 and 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, however, these sources 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.

The hepatitis C virus (HCV) is known to be the causative agent for most blood-borne non-A, non-B hepatitis (NANBH). Studies throughout the world indicate that HCV is transmitted through contaminated blood and blood products, through blood transfusions or through other close, personal contacts. The presence of anti-HCV indicates that a person may have been infected with HCV; the presence of HCV RNA indicates that the person may be infected currently and be capable of transmitting HCV infection. In the context of NHANES, an HCV RNA positive result likely indicates chronic infection with hepatitis C virus.

HCV infection is the most common chronic blood-borne infection in the United States with an estimated seroprevalence of 1.7%. An estimated 2.4 million Americans suffer from chronic HCV infection making it the leading cause of chronic liver disease. It is estimated that >50% of those with acute hepatitis C progress to chronic infection, that can result in cirrhosis, liver cancer and death. In the United States, chronic hepatitis C (CHC) was responsible for an estimated 14,000 deaths in 2019

and is the leading cause of liver transplantation. The burden of HCV-associated disease is likely to increase during the next 10 to 20 years as the infected cohort reaches an age at which complications of liver disease typically occur. Hepatitis C infection among younger adults has increased as a consequence of the opioid and infectious diseases syndemic, placing new generations at risk, including infants born to pregnant persons with hepatitis C infection.

In 2013, CDC revised its guidelines for Hepatitis C (HCV) testing because of 1) changes in the availability of certain commercial HCV antibody tests; 2) evidence that many persons who are identified as reactive by an HCV antibody test might not subsequently be evaluated to determine if they have current HCV infection; and 3) there have been significant advances in the development of antiviral agents with improved efficacy against HCV (Moyer, 2013). The 2020 revised guidelines recommended testing at least once for everyone aged 18 years and older, and pregnant persons during every pregnancy (Schillie, 2020).

In 2013, there was a change to the HCV testing algorithm. The flow chart for the HCV testing algorithm can be found in the laboratory method file or by following the MMWR link: https://www.cdc.gov/mmwr/pdf/wk/mm62e0507a2.pdf.

Samples found reactive to the HCV antibody screening test are tested for HCV RNA. All HCV RNA positive samples were tested for HCV genotype, and only HCV RNA negative samples were tested with an HCV antibody confirmation test.

The NHANES viral hepatitis laboratory component includes an HCV antibody screening test, followed by an HCV RNA test for antibody screening reactive samples. HCV RNA positive samples are genotyped, and HCV RNA negative samples are tested to confirm the antibody screening result.

Examined participants aged 6 years and older in the NHANES 2017-March 2020 pre-pandemic sample were eligible for the HCV antibody screening, HCV RNA, HCV genotype, and HCV antibody confirmation tests.

Test Principle:

The VITROS Anti-HCV test is performed using the VITROS Anti-HCV Reagent Pack and VITROS Immunodiagnostic Products Anti-HCV Calibrator on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System.

An immunometric technique is used. This involves a two-stage reaction. In the first stage, HCV antibody present in the sample binds with HCV recombinant antigens coated on the wells. Unbound sample is removed by washing. In the second stage, horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-human IgG) binds to any human IgG captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing

light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indicative of the level of anti-HCV present in the sample.

Three recombinant hepatitis C virus encoded antigens (c22-3, c200 and NS5) are used in the VITROS Anti-HCV test. The recombinant protein c22-3 is encoded by the putative core region of the HCV genome. HCV recombinant protein c200 is encoded by the putative NS3 and NS4 regions of the HCV genome. The c200 protein contains the c33c protein sequence which is genetically linked to the c100-3 protein sequence. Studies have indicated that antibodies which develop after infection with HCV are often reactive with c22-3 and/or c33c HCV recombinant protein NS5 is encoded by the putative NS5 region of the HCV genome. A significant proportion of persons infected with HCV develop antibodies to NS5.

The host organism for all three HCV recombinant antigens is S. cerevisiae (yeast).

2. SAFETY PRECAUTIONS

Test kits 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, test kit components should be treated as though they are capable of transmitting HCV or other infectious agents. Consider all serum specimens for analysis potentially positive for infectious agents including HIV, hepatitis B virus and HCV. Controls and samples 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.

Observe universal precautions when performing the assay, thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water, handle samples with extreme care to prevent sample contamination, wear personal protective apparel, disposable gloves and eyewear during all steps of this method to minimize both infectious and chemical contamination hazards.

Do not eat, drink, smoke, or apply cosmetics in areas where reagents or samples are handled. If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Do not use components beyond the expiration date on the kit. Alterations in the physical appearance of kit components may indicate instability or deterioration. Do not mix reagents from different lots. We recommend Biosafety Level 2 containment procedures as described in CDC/NIH publication #93-8395[i] be used by those handling test specimens and kit reagents.

Use all pipetting devices and instruments with care and follow the manufacturer’s instructions for calibration and quality control.

Risk is minimal due to the small quantity of chemicals, the safety of packaging and the limited handling by the operators using the test kits.

3. COMPUTERIZATION; DATA MANAGEMENT SYSTEM

The Data Management System (DMS) was used through December 31, 2019.

The run information can be uploaded into the computerized database (DMS) after the run information is exported by the software. 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 through December 31, 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 1, 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 the ELIMS at CDC is available on an intranet site available to laboratory staff.

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

No special patient preparation is necessary.

Specimens Recommended: Serum

Do not use turbid specimens. Turbidity in specimens may affect test results.

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-HCV test uses 20 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. Handle samples in stoppered containers to avoid contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or crosscontaminating sample tube stoppers.

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.

The National Committee for Clinical Laboratory Standards (NCCLS) 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).
– If the test will not be completed within 48 hours, or for shipment, freeze samples at -20°C (-4°F) or colder.

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. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

a. Instrumentation and Software

VITROS ECi/ECiQ Immunodiagnostic Systems

VITROS 3600 Immunodiagnostic System

b. Reagents

For the *in vitro* qualitative detection of immunoglobulin G antibody to hepatitis C virus (anti-HCV) in human serum and plasma (heparin, EDTA and sodium citrate) using the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System. Three recombinant hepatitis C virus encoded antigens are used.

VITROS Immunodiagnostic Products Anti-HCV Reagent Pack

VITROS Immunodiagnostic Products Anti-HCV Calibrator

Reagent Pack Contents 1
reagent pack containing:

- 100 coated wells [Hepatitis C virus recombinant antigens (NS5, c22-3, c200) derived from yeast (S. cerevisiae); coated at 0.41 µg/well]
- 18.2 mL assay reagent (buffer with 2-chloroacetamide anti-microbial agent)
- 20.6 mL conjugate reagent (HRP-mouse monoclonal anti-human IgG, 1.04 ng/well) in buffer with anti-microbial agent (1% ProClin 300 w/w)

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	≤8 weeks
Opened	Refrigerated	2–8°C (36–46°F)	≤8 weeks

- The VITROS Anti-HCV 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.

c. *Calibrators*

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems and the VITROS 3600 Immunodiagnostic System for the *in vitro* qualitative detection of immunoglobulin G antibody to hepatitis C virus (anti-HCV) in human serum and plasma (heparin, EDTA and sodium citrate) using VITROS Anti-HCV Reagent Packs. The VITROS Anti-HCV Calibrator has been validated for use only on the VITROS ECi/ECiQ

Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System with the VITROS Immunodiagnostic Products Anti-HCV Reagent Pack.

Calibrator Contents

- 1 VITROS Anti-HCV Calibrator (anti-HCV positive human plasma in anti-HCV negative human plasma with antimicrobial agent, 2 mL)
- Lot calibration card
- Protocol card
- 8 calibrator bar code labels

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 determinations of the calibrator.
- 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.

Calibrator Storage and Preparation

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

- VITROS Anti-HCV Calibrator is supplied ready for use.
- The VITROS Anti-HCV Calibrator is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Opened calibrators may be stored frozen (with no more than 1 freeze-thaw cycle).
- Evaporation will occur when calibrators are stored open on the system. For more information, refer to the operating instructions for your system.
- The VITROS Anti-HCV test uses 20 µL of calibrator for each determination. The VITROS Anti-HCV Calibrator may be used directly on the VITROS Immunodiagnostic and VITROS Integrated Systems. Alternatively, transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.
- The VITROS Anti-HCV Calibrator is automatically processed in duplicate.

d. *Materials Required but not Provided*

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials such as VITROS Immunodiagnostic Products Anti-HCV Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration

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 the calibrator in the same manner as samples. Load sufficient for the automatic duplicate determination. Calibration need not be programmed if bar code labels are used; 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 (see System Operator's Guide).
- 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-HCV 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 is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic Systems.

b. Verification

Not Applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) The VITROS aHCV – Anti-HCV Reagent Pack is used for 100 tests. Reagent pack is supplied ready for use and its components cannot be interchanged within a manufacturer's lot or between lots.

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

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

(4) Prepare a runsheet listing controls and specimens in the order presented in the e-file.

(5) Perform daily maintenance of the VITROS ECi and V3600 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 and control specimens from the respective cold storage unit (freezer or refrigerator) 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 Manual using the VITROS Anti-HCV Reagent Pack and associated controls and calibrator packs. Do not use expired reagents.

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

a. Take off and discard screw caps from the cryo-vials, 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 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 runsheet were scanned by the instrument before the test begins. If a barcode cannot be scanned due to incorrect positioning or an unreadable label, enter the 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 to CDC are accessioned by the Specimen Triage and Tracking Team (STATT) into ELIMS for Unit 90 under CLIA regulated practices, and then Unit 90 is notified.
b. Testing Personnel 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

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.

d. Reporting results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

e. Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS AntiHCV test upon completion of all testing steps required in the testing algorithm

Final VITROS Anti-HCV Test Result (s/c)	**Conclusion from Testing Algorithm**	**Interpretation**
<1.00	Negative	Anti-HCV IgG not detected. Patient is presumed not to be infected with HCV.
≥1.00	Reactive	Anti-HCV IgG detected. Patient is presumed to be infected with HCV, state or associated disease not determined. Follow CDC recommendations for supplemental testing.*

* CDC. Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians. MMWR, May 10, 2013; 62(18):362-365.

- Results obtained with the VITROS Anti-HCV test may not be used interchangeably with values obtained with different manufacturer's test methods.
- The magnitude of a VITROS Anti-HCV test result cannot be correlated to an endpoint titer.

- Citrated plasma has been shown to lower the signal/cutoff (s/c) values in some anti-HCV reactive samples. High negative results (0.80–0.99 s/c) obtained on samples collected with this anticoagulant should be interpreted accordingly. Additional testing may be required. Follow manufacturer's instructions for using plasma collection containers with anticoagulants.

f. Recording of Data

The system automatically determines the HCV titer for the sample or control.

If **Negative Control or Positive Control** is invalid, then the entire run is invalid; repeat the entire run including control and samples. A valid run may include both valid and invalid sample results.

g. Calculations

Results are calculated as a normalized signal, relative to the 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 VITROS Integrated Systems.

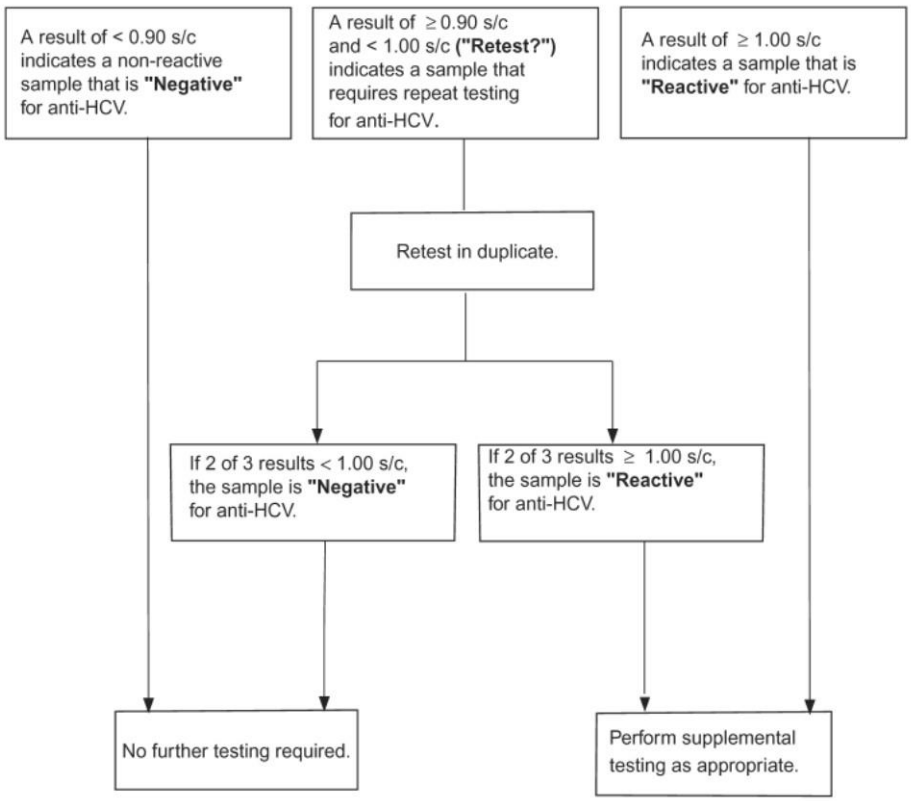
$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

Patient sample results will be displayed with a **"Negative"**, **"Retest?"**, or **"Reactive"** label. An initial result labeled with **"Retest?"** indicates a sample that requires repeat testing for antiHCV.

Result (s/c)	<0.90	≥0.90 and <1.00	≥1.00
Result Text	Negative	Retest?	Reactive

Final results should be manually interpreted using the algorithm below.

Testing Algorithm



9. REPORTABLE RANGE OF RESULTS

Calculated results of <0.90 s/c upon initial testing or 2 of 3 tests with <1.00 s/c upon repeated testing are non-reactive and the sample is reported as negative for anti-HCV confirmed antibody. Calculated results of ≥1.00 s/c upon initial testing or 2 of 3 tests with ≥1.00 s/c upon repeated testing are reactive and supplemental testing is performed to confirm the presence of HCV antibody before the sample result for anti-HCV confirmed antibody is reported.

10. QUALITY CONTROL (QC) PROCEDURES

Quality Control Material Selection

VITROS Anti-HCV Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. There are 2 VITROS Anti-HCV Controls (Anti-HCV negative and Anti-HCV 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-HCV methods if they contain high concentrations of preservatives, stabilizers, or other nonphysiological 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-HCV test.

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-HCV decision point (signal/cutoff [s/c] $\geq$1.00).

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

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 or maintenance to critical parts or subsystems that might influence performance of the test (see System Operator's Guide)

If 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 advisable to repeat some or all patient specimens before reporting results for this run.

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

The entire run is considered to be invalid if one or both controls are not within specified limits.

Repeat the entire test process: specimen and control preparation, reverse transcription, amplification and detection.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

The results from this or any other diagnostic test 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 or infection with HCV. HCV antibodies may be undetectable in some stages of the infection and in some clinical conditions.

Follow CDC recommendations for supplemental testing of reactive samples.

Results from immunosuppressed individuals should be interpreted with caution.

The prevalence of the analyte will affect the test's predictive value.

Test performance characteristics have not been established for any other specimen matrices than serum or heparin, EDTA and sodium citrate anticoagulated plasma.

Heterophilic, e.g. human anti-mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays. These antibodies may be present in blood samples

from individuals regularly exposed to animals or who have been treated with animal serum products.
The cross-reactivity of the VITROS Anti-HCV test with other flaviviruses known to cause hepatic disease has not been established.

The cross-reactivity of the VITROS Anti-HCV test with antibodies to S. cerevisiae has not been established.

13. REFERENCE RANGES (NORMAL VALUES

All normal, noninfected humans should have negative values for HCV confirmed antibody.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25°C (68-77°F) during preparation and testing only.

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

Other FDA-licensed tests for anti-HCV may be substituted but must be accompanied by validation data to show substantial equivalence with this assay. Substitution of test methods may not be done without approval from the NCHS.

Alternate storage is not recommended.

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 DATA

Since hepatitis C antibody (confirmed) is qualitative data there are no summary statistics or QC graphs.

REFERENCES

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Confirmatory test – Fujirebio INNO-LIA HCV Score Assay

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical Relevance:

The INNO-LIA HCV Score is a Line Immuno Assay (LIA) for the detection of antibody to hepatitis C virus (anti-HCV) in human serum or plasma. It is intended for use as a supplementary test on human serum or plasma specimens found to be reactive using an anti-HCV screening procedure. For research use only. Not for use in diagnostic procedures.

Test Principle:

The INNO-LIA HCV Score is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), the NS3 helicase region, and the NS4A, NS4B and NS5A regions. The antigens are coated as 6 discrete lines on a nylon strip with plastic backing. In addition, four control lines are coated on each strip: streptavidin control line, 3+ positive control (anti-human Ig) which is also used as sample addition control line, 1+ positive control (human IgG), and the ± cut-off line (human IgG).

The INNO-LIA HCV Score is based on the principle of an enzyme immunoassay. A test sample is incubated in a trough together with the test strip. If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip. Subsequently, an affinity-purified alkaline phosphatase-labeled goat anti-human IgG (H+L) conjugate is added and reacts with specific HCV antigen/antibody complexes, if previously formed. Incubation with the enzyme substrate produces a chestnut-like color, the intensity of which is proportional to the amount of HCV-specific antibody captured from the sample on any given line. Color development is stopped with sulfuric acid.

2. SAFETY PRECAUTIONS

Specimens, positive control and negative control should always be handled as potentially infectious. The positive control has been found to be negative for anti-HIV and HBsAg and the negative control has been found to be negative for anti-HIV-1/HIV-2, anti-HCV and HBsAg. No test method can offer complete assurance that blood products will not transmit infectious agents. Therefore, all blood components and biological materials should be considered as being potentially infectious and handled as such.

Only adequately trained personnel should be permitted to perform the test procedure.

All blood components and biological materials should be disposed of in accordance with established safety procedures.

- Autoclave for at least 15 minutes at 121°C.
- Incinerate disposable material.
- Mix liquid waste with sodium hypochlorite so that the final concentration is ±1% sodium hypochlorite. Allow to stand overnight before disposal. CAUTION: Neutralize liquid waste that

contains acid before adding sodium hypochlorite. Do not aspirate the stop solution into a bottle which contains sodium hypochlorite.

Waste should be handled according to the institution's waste disposal guidelines. All federal, state and local environmental regulations should be observed.

Use of personal protective equipment is necessary. Observe universal precautions; wear protective gloves, eyewear, and lab coat during all steps of this method because of both infectious and chemical contamination hazards.

Sample diluent contains 2-Chloroacetamide to which the following hazard and precautionary statements apply: May cause an allergic skin reaction. Wear protective gloves, protective clothing eye protection, face protection. Avoid breathing mist/vapors/spray. IF ON SKIN: wash with plenty of soap and water.

Substrate contains N,N-Dimethylformamide to which the following hazard and precautionary statements apply: May damage the unborn child. Use personal protective equipment as required.

3. COMPUTERIZATION; DATA MANAGEMENT SYSTEM

Raw data are transcribed manually into a computerized database.

The Data Management System (DMS) was used through December 31, 2019.

The run information can be uploaded into the computerized database (DMS) after the run information is extracted by the software. 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 31, 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 1, 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 ELIMS database, are archived after 12 months and can be retrieved any time upon request.

All necessary information about the ELIMS at CDC can be found on an intranet site accessible by laboratory staff.

The intensity of the reaction on the control lines on each strip is used to assign the reactivity ratings for each the 6 antigen lines on that strip. Results are summarized in the Global Result analyte and expressed as "positive", "negative" or “indeterminate”.

Other information in the database may typically include the CDC sample identification number (CSID), CDC Unique ID (CUID), the CDC Local ID (NHANES specimen ID), the date collected, the date tested and results of testing for other hepatitis markers.

Both DMS-generated and ELIMS-generated NHANES reports are reviewed by the laboratory Technical Supervisor and transmitted to the CDC Division of Viral Hepatitis Epidemiology and Surveillance Branch for secondary QC and subsequent submission to NCHS along with other NHANES data.

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

Specimens submitted for testing are handled according to the Hepatitis Reference Laboratory Standard Operating Procedure entitled "Sample Handling" (S. Kamili, J. Drobeniuc; 06/2008).

No special instructions such as fasting or special diets are required. Diurnal variation is not a major consideration.

Specimens may be serum, recalcified plasma, or plasma. Serum specimens may be collected using regular red-top or serum-separator Vacutainers.

Required sample volume is 10 µL for the assay; 1.0 mL will permit repeat analyses as well as other testing.

Specimens should be stored in plastic vials and sealed tightly to prevent desiccation of the sample.

Serum or plasma samples are collected aseptically to minimize hemolysis and bacterial contamination.

Samples are stored in labeled 2 mL Nalgene cryovials or equivalent. H. Serum can be stored refrigerated (2°C-8°C [36–46°F]) for up to 7 days, frozen (≤-20°C [-4°F]) for 7 days or longer, and frozen at ≤ -60°C (≤-76°F) for 12 months or longer; and can be subject to three (3) freeze/thaw cycles.

Samples held in long-term storage at < -60°C (≤-76°F) for the NHANES cycles through December 31, 2019, were indexed in the NHANES Index spreadsheet for easy retrieval; For the specimens from cycles starting on January 1, 2020, all the information is available from ELIMS.

Specimens are rejected if contaminated, hemolyzed, or improperly labeled and stored. However, specimens are rejected only after consultation with NCHS.

Do not use heat-activated specimens.

Performance has not been established for cadaver specimens or body fluids other than serum or plasma (such as urine, saliva or pleural fluid.)

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

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

A. Instrumentation

(1) Orbital shaker or rocker.

(2) Vortex mixer

(3) Gilson Pipetman micropipettors, 10 µL 20-200 µL and 200-1000 µL sizes (Rainin Instrument Co., Woburn, MA).

(4) Vacuum aspirator

(5) Timer

B. Materials

(1) Deionized water (Continental Water Systems, Inc., San Antonio, TX).

(2) Pipet tips, (Rainin Instrument Co.).

(3) Protective gloves, Tronex or Flexam, small/medium/large (Best Manufacturing, Menlo Park, GA).

(4) 2 mL cryovials, cat. no. 5000-0020 (Nalge Company, Inc., Rochester, NY).

(5) Cryovial boxes, cat. no. 5026-0909 (Nalge Company, Inc., Rochester, NY).

(6) Graduated cylinders; 10, 25, 50 and 100 ml.

(7) Forceps for handling strips (any vendor).

C. Reagents

Reagents/Materials supplied:

(1) Strips (20) Containing 20 INNO-LIA™ HCV antigen-coated test strips.
(2) Sample Diluent (30 ml) Containing color-coded (green) phosphate buffer containing sodium chloride, detergent, bovine protein stabilizers and 0.3% chloroacetamide (CAA) as preservative.
(3) Ready-to-use Conjugate (45 ml) Containing color-coded (red) goat anti-human IgG labeled with alkaline phosphatase in Tris buffer containing bovine stabilizers, detergent and 0.01% methylisothiazolone (MIT)/0.1% CAA as preservative.
(4) Negative Control (0.12 ml) Containing basematrix of human origin with 0.01% MIT/0.1% CAA as preservative.
(5) Positive Control (0.12 ml) Containing inactivated human serum positive for antibodies to HCV with 0.01% MIT/0.1% CAA as preservative.
(6) Ready-to-use BCIP/NBT Substrate (45 ml) Containing 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium in dimethyl formamide, with 0.01% MIT/0.1% CAA as preservative.
(7) Stop Solution (45 ml) Containing 0.1 mol/l sulfuric acid.
(8) Wash Solution (45 ml) Containing color-coded (blue) Tris buffer containing sodium chloride, detergent and 0.02% bromo-nitro-dioxane as preservative, to be diluted 5x in distilled water. Diluted wash solution is stable for 2 weeks if kept at 2 - 8°C (36–46 °F).
(9) Incubation tray (2) With 11 troughs each.
(10) Adhesive sealers (5)
(11) Data reporting sheet (1) For storage of developed strips.
(12) Reading card (1) For identification of reactive antigen lines.

D. Reagent Preparation and Storage
(1) If kept at 2 - 8°C (36–46 °F), opened or unopened, all reagents are stable until the expiration date. Do not freeze reagents.
(2) Do not use the kit beyond the expiration date.
(3) All reagents and the plastic tube containing the test strips must be brought to room temperature (18 - 25°C [64-77°F]) approximately 30 minutes before use and returned to the refrigerator (2 - 8°C [36–46 °F]) immediately after use.
(4) Alterations in the physical appearance of kit reagents may indicate instability or deterioration.
(5) After opening the original tube containing the strips, any unused strip will be stable for 16 weeks if stored at 2 - 8°C (36–46 °F) in the closed original tube with desiccant.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve
No calibration curve is generated by the user as part of these assay methods.

B. Verification
Not Applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1) Do not mix reagents with different lot numbers.

2) Frozen reagents, e.g., stored too close to cooling element, can cause erroneous results.

3) Make sure correct sample volume and washing times are used for the test procedure.

4) The positive and negative controls should be assayed with each series of patient/donor specimens. The positive and negative controls should be treated exactly as patient/donor specimens throughout the assay procedure.

5) Avoid microbial contamination of reagents.

6) Ensure that the samples and controls are homogeneous before use.

7) Do not touch the membrane of the strip. Always manipulate the strips with the plastic backing.

8) Use a new pipette tip for each specimen.

9) Make sure that the test strips are placed in the troughs with their membrane side facing upwards.

10) All incubation steps should be performed using an orbital shaker or rocker. Use rocker only for overnight sample incubation.

11) The shaking of the solutions over the strip is important in achieving even line staining and maximum sensitivity. During shaking the strip surface should be completely submerged.

12) Cover the troughs with an adhesive sealer to avoid drying of the strips during the overnight sample incubation.

13) Unused and developed strips should be kept away from strong light and heat.

14) The kit should be used by personnel trained in clinical laboratory practices.

15) Re-use of strips or troughs will result in erroneous results.

16) Cutting strips will result in erroneous interpretation of the results.

B. Sample Preparation

1) Bring serum specimens to 20-25ºC (68-77ºF). While one box or rack of samples is being pipetted, the other racks should be refrigerated.

2) Serum and plasma samples may stratify when frozen or stored at 4-8ºC (39-46 ºF) for extended periods. Mix specimens gently before testing.

C. Manual Test Procedure

- 16 hours sample incubation

1) Use the required amount of test troughs, taking into account that for each test run, a Positive and a Negative Control should be assayed. Identify test troughs as controls and specimens, and place them in the tray.

2) Add 1 ml of Sample Diluent to each test trough.

3) Add 10 µl of the appropriate specimen or control to the appropriately labelled trough.

4) Remove the required amount of test strips from their container, and add one strip to each of the test troughs. The test strip is placed membrane side upwards into the trough using tweezers. THE STRIP MUST BE COMPLETELY SUBMERGED.

5) Cover the troughs with an adhesive sealer (see Remarks and precautions). Incubate the samples by placing the tray on a shaker or rocker (see Direction for incubation) and agitate OVERNIGHT (16 ± 2 h) at room temperature (18 - 25°C [64-77°F]). NOTE: Carefully remove the adhesive sealers to avoid cross-contamination.

6) Wash each test strip 3 times (5 minutes) with 1 ml Wash Solution (see Directions for washing).

7) Add 1 ml of Conjugate Solution to each test trough.

8) Incubate with the conjugate by placing the test tray on the shaker or rocker and agitate for 30 minutes at room temperature (18 - 25°C [64-77°F]).

9) Wash each test strip 3 times (5 minutes) with 1 ml Wash Solution (See Directions for washing).

10) Add 1 ml of Substrate Solution to each test trough.

11) Incubate with the substrate by placing the test tray on the shaker or rocker, and agitate for 30 minutes at room temperature (18 - 25°C [64-77°F]).

12) Aspirate liquid. Add 1 ml of Stop Solution to each trough.

13) Incubate with the stop solution by placing the test trough on the shaker or rocker, and agitate for 10 - 30 minutes at room temperature (18 - 25°C [64-77°F]).

14) Aspirate Stop Solution.

15) Remove the strips from the test troughs and place them, membrane side upwards, on absorbent paper using tweezers. As soon as the strips have dried completely, results can be interpreted. To accelerate the drying process, place strips in a dry incubator at 37°C [98.6°F] for 30 minutes or use a hot air fan for 1 minute.

Developed strips will retain their color if stored in the dark.

- ***2 and 3 hours sample incubation***

For the "2 hours and 3 hours sample incubation" protocol the same 15 steps as for the test procedure "16 hours sample incubation" will be followed, but changes to steps 3 - 5 - 6 and 9 have to be taken into account. Sample volume for specimens and controls will increase from 10 to 20 µl (step **3**) and sample incubation time changes to 2 and 3 hours (step **5**). Washing after sample incubation changes for the 2 hours procedure to 3 times 10 minutes and for the 3 hours procedure to 3 times 6 minutes (step **6**); finally the second washing is 3 times 3 minutes for the 2 and 3 hours sample incubation (step **9**).

NOTE: For NHANES, the 16-hour sample incubation is used.

Directions for washing

1) After overnight, 2 hour or 3 hour incubation, carefully remove the adhesive plate sealer
2) The liquid is aspirated from the trough with a pipette, preferentially attached to a vacuum aspirator, which contains 5% sodium hypochlorite solution in the waste bottle.
3) The tray is held at an angle to allow all fluid to flow to one side of the trough (to the uncoated plastic backing part of each strip).
4) Add 1 ml of diluted wash solution to each trough and agitate on a shaker or rocker. Shaking time is indicated in the assay procedure.
5) Repeat these steps as many times as indicated in the assay procedure. 6) Do not allow the strips to dry between the washing steps.
7) Make sure not to damage the surface of the test strip when aspirating.
8) Always use a clean aspiration device with disinfectant trap to avoid cross-contamination.
9) Make sure the entire strip is thoroughly washed by complete submersion in the washing solution.
10) Adapt the speed of the shaker or rocker when necessary.
11) Avoid splashing of the wash solution over the edges of the troughs.

Directions for incubation

1) All the incubation steps (sample, conjugate, substrate and stopping solution incubation) and the washing steps should be performed on a shaker or rocker. Use rocker only for overnight sample incubation.

2) During incubation and washing steps, the strip surface should be completely submerged with the membrane side facing upwards.
3) The shaker or rocker should allow a reciprocal (to-and-fro) motion of the strips in the trough, and movement of the liquid over the strips without spilling over the trough.
4) The speed generated by an orbital or longitudinal shaker or rocker is critical in achieving even line staining and maximum sensitivity

D. Recording of Data

A NEGATIVE, INDETERMINATE, or POSITIVE interpretation is based on the reaction pattern present on the strip. The intensity of the reaction on the ±, 1+ and 3+ control lines on each strip is used to assign the reactivity ratings for each antigen on that strip:

Intensity of antigen line reaction	**Rating**
Less than ±	-
Equal to ±	±
More than ± but less than/equal to 1+	1+
More than 1+ but less than 3+	2+
Equal to 3+	3+
More than 3+	4+

A reactivity rating must be made separately for each strip. Use the reading card for correct interpretation. Identification of the lines is obtained by alignment of the 3+ control line on the developed strip with the corresponding 3+ control line on the reading card.

Interpretation of Results

All HCV antigen lines have a negative reactivity rating Or Only 1 HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3	NEGATIVE
A single HCV antigen line has a reactivity rating of 1+ or higher Or The NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative	INDETERMINATE

At least 2 HCV antigen lines have a reactivity of ± or higher	POSITIVE

A POSITIVE test result indicates the presence of anti-HCV and past or present anti-HCV infection.

An INDETERMINATE test result indicates that anti-HCV may or may not be present and that a decision as to whether past or present HCV infection exists cannot be made. Since reactivity of 1+ or greater to any of the virus-encoded antigens on the strip is possible evidence of past or present HCV infection, all individuals who are INDETERMINATE should be retested again over a period of 6 to 12 months to ascertain whether increased reactivity has occurred. It is recommended that individuals who are INTEDETERMATE be retested after six months using a freshly drawn specimen.

A NEGATIVE test result which is REACTIVE by a licensed anti-HCV screening procedure does not exclude the possibility of infection with HCV. Levels of anti-HCV may be undetectable during the early stages of infection.

9. REPORTABLE RANGE OF RESULTS

Samples on which INNO-LIA testing is performed have confirmed anti-HCV antibody reported as positive, negative or indeterminate based on the INNO-LIA test result. Samples on which screening antiHCV results were negative are reported as negative for confirmed anti-HCV antibody.

10. QUALITY CONTROL (QC) PROCEDURES

Check the validity of the positive and negative control strips and the validity of the control levels on each strip before reading the test results.

A. Validation of the test run

- The positive control strip must show a reaction of at least 1+ on the C1, C2, NS3 and NS4 antigen lines. The E2 and NS5 antigen lines mat show a negative rating.
- The negative control strip must show a negative rating (no reaction at all or at least less than control level ± for all the antigen lines).

B. Validation of a single strip

- The control levels 1+ and ± as well as the strong positive control level 3+ should be visible.
- The intensity of the control level 3+ should be greater than that of level 1+ and the intensity of the level 1+ should be greater than that of level ±.
- The control line for anti-streptavidin should have a negative rating.

If the assay does not meet the criteria above, then the run is invalidated and must be repeated.

Note:

- The strip must be completely dried to avoid any misinterpretation due to faintly visible bands appearing after addition on stop solution
- Do not place paper on top of the strips as long as they are wet.
- Weak control bands can be observed for samples containing high IgG levels (above the normal range).

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

Repeat run for individual sample as described above.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

The sample is restricted to human serum or plasma. No interfering substances are identified. The protocol provided must be strictly followed to obtain optimal performance of the assay. Do not use heat-activated specimens. Samples with a single $\pm$ reactivity or higher on NS3 can be indicative of HCV seroconversion; they are therefore scored as indeterminate. If an indeterminate result is obtained, it is recommended to test an additional sample after a few weeks. A negative test does not exclude the possibility of exposure to or infection with HCV. Negative results in this assay in individuals with prior exposure to HCV or early infection may be due to antibody levels below the limit of detection of the assay or lack of antibody reactivity to the HCV antigens used in this assay. A sample giving a positive reaction on the streptavidin control line may give cross-reactions with other HCV antigen lines and cannot be determined as positive for HCV antibodies. In a hemodialysis setting, antibodies may be undetectable. The use of diluted samples may give erroneous results.

13. REFERENCE RANGES (NORMAL VALUES)

All normal non-infected humans should have negative values for antibodies to hepatitis C virus.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25ºC (68-77ºF) during preparation and testing for up to 4 hours.

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

Other FDA-licensed tests for confirmation of total anti-HCV may be substituted but must be accompanied by validation data to show substantial equivalence with this assay. Substitution of test methods may not be done without approval from the NCHS.

Alternate storage is not recommended.

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. Plots of OD values are not generated for quality control purposes.

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