

# 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  
Chiron RIBA HCV Version 3.0 Strip Immunoblot Assay Kit

*Method No.:*

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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](mailto:sek6@cdc.gov)

## Important Information for Users

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

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**Public Release Data Set Information**

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

<b>Lab Name</b>	<b>Variable Name</b>	<b>SAS Label</b>
HEPC_G	LBDHCV	Hepatitis C antibody (confirmed)

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

### **1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE**

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.

The hepatitis C virus (HCV) is now known to be the causative agent for most, if not all, 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 an individual may have been infected with HCV and may be capable of transmitting HCV infection.

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.

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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<sup>i</sup> 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 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.

Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES IV data.

Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly by CDC Data Center staff.

Documentation for data system maintenance is maintained with printed copies of data records for 2 years.

### 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.

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The VITROS Anti-HCV test uses 20  $\mu$ L 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 cross-contaminating 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 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. 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

VITROS 5600 Integrated 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 and the VITROS 5600 Integrated 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:

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- 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, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated 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*

<b>Calibrator</b>	<b>Storage Condition</b>		<b>Stability</b>
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)

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- 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 and VITROS Integrated 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; do not freeze.

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- (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 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, than 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 runsheet.
- (3) 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.
- (4) After completion of the test, interface DMS with the VITROS instrument and import the results into the DMS.

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

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The following table summarizes the interpretation of results obtained with the VITROS Anti-HCV 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. *Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-Related Chronic Disease*. MMWR 1998;47 (RR-19)

- 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 anti-HCV.

<b>Result (s/c)</b>	<0.90	≥0.90 and <1.00	≥1.00
<b>Result Text</b>	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  $\geq 1.00$  s/c upon initial testing or 2 of 3 tests with  $\geq 1.00$  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.

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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 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**

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

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## Confirmatory test - Chiron RIBA

### 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Chiron RIBA HCV 3.0 Strip Immunoblot Assay (SIA) is an *in vitro* qualitative enzyme immunoassay for the detection of antibody to hepatitis C virus (anti-HCV) in human serum or plasma. Detection of anti-HCV by SIA methodology is based upon traditional Western and dot blotting techniques, in which specific immunogens (i.e. antigenic polyproteins) encoded by the HCV genome are immobilized onto a membrane support. Visualization of anti-HCV reactivity in specimens to the individual HCV-encoded proteins is accomplished using anti-human IgG enzyme-conjugates in conjunction with a colorimetric enzyme substrate. Qualitative determination of the human antibody directed against hepatitis C virus (anti-HCV) in human serum or plasma is measured using direct solid-phase enzyme immunoassay.

### 2. SAFETY PRECAUTIONS

Test kits for the strip immunoblot assay 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. Consider all serum specimens for analysis potentially positive for infectious agents including HIV, hepatitis B virus and HCV. Observe universal precautions; wear protective gloves, eyewear, and lab coat during all steps of this method because of both infectious and chemical contamination hazards. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. We recommend Biosafety Level 2 containment procedures as described in CDC/NIH publication #93-8395 be used by those handling test specimens and kit reagents .

Material safety data sheets for sodium azide, sulfuric acid, hydrochloric acid, o-phenylenediamine, and sodium hypochlorite are available through the National Center for Infectious Diseases computer network. 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

A. Raw data are transcribed manually from an instrument readout sheet into a computerized database or it can be uploaded in to the computerized database from a disk after the run information is exported from the instrument. 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 cutoff value calculated from the controls. Results are expressed as "positive" or "negative" or "indeterminate" for RIBA. Other information in the database may typically include the HRL 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. Electronically stored data are backed up routinely.

B. Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES IV data.

C. Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly by CDC Data Center staff.

D. Documentation for data system maintenance is maintained with printed copies of data records for 2 years.

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

A. Specimens submitted for testing are handled according to the HRL SOP entitled "Sample Handling" (S. Kamili, J.Drobeniuc; 06/2008).

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

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

D. Required sample volume is 20 µL for the assay; 1.0 mL will permit repeat analyses as well as other testing.

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

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

G. Samples are stored in labeled 2 mL Nalgene cryovials or equivalent.

H. Serum is best stored frozen, and freeze/thaw cycles should be kept to a minimum. Store samples at 4-8°C for no more than 5 days.

I. For storage >5 days, samples are held at -20°C. Samples held in long-term storage at -70°C are indexed in the database for easy retrieval.

J. Specimens are rejected if contaminated, hemolyzed, or stored improperly. However, rejection is done only after consultation with NCHS.

K. Avoid multiple freeze/thaw cycles.

L Do not use heat-activated specimens.

M. 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

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- (1) RIBA Processor System (Chiron Corporation, Emeryville, CA).
- (2) RIBA Processor System Installation Kit Chiron Corporation, Emeryville, CA).
- (3) Gilson Pipetman micropipettors, 10 and 200 :L sizes (Rainin Instrument Co., Woburn, MA).

B. Materials

- (1) Deionized water (Continental Water Systems, Inc., San Antonio, TX).
- (2) Pipet tips, cat. nos. RT20 & RT200 (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) 1.5 mL microtubes (Marsh Biomedical Products, Rochester, NY).
- (7) 50 mL-polypropylene tubes (Corning Glass Works, Corning, NY).
- (8) Fixed or adjustable pipetting devices capable of delivering 20 ml and 1000 ml with at least +/- 5% accuracy (Gilson Pipetmen models P-20 and P-1000).
- (9) Forceps for handling strips (any vendor).
- (10) Chiron RIBA HCV 3.0 Strip Immunoblot Assay, Hepatitis C Virus Encoded Antigen/Peptide (Recombinant c33c and NS5 antigens; Synthetic 5-1-5, c100, and c22 peptides), cat. no. 930740 (Ortho Diagnostic Systems, Inc. Raritan, NJ).
- (11) RIBA Processor System (Ortho Diagnostic Systems, Raritan, NJ; product code 936595).
- (12) RIBA Processor System Installation Kit.

C. Reagents

Chiron RIBA HCV Version 3.0 Strip Immunoblot Assay kits contain the following reagents; prepared by the manufacturer. Volumes listed are for 30 tests.

- (1) Hepatitis C virus encoded antigen/peptide (Recombinant c33c and NS5; Synthetic 5-1-1p, c100p, and c22p) Coated strips

Each strip contains four individual bands coated with HCV-encoded antigens/peptides, a recombinant human SOD band, and two IgG control bands. 30 Consecutively numbered strips are provided. The automated version contains 3 sealed pouches, each with 10 strips in reaction vessels.

- (2) Conjugate

1 bottle (175 mL). Peroxidase-labeled goat anti-human IgG (heavy and light chains), with bovine protein stabilizers. Preservative: 0.01% thimerosal.

(3) Specimen diluent

1 bottle (175 mL). Phosphate-buffered saline with bovine protein stabilizers and detergents. Preservative: 0.1% sodium azide and 0.05% gentamicin sulfate.

(4) Substrate solution

1 bottle (17 ml). 4-chloro-1-naphthol-in methanol.

(5) Substrate buffer

1 bottle (90 mL). Phosphate-buffered hydrogen peroxide.

(6) Wash Buffer Concentrate (50X)

1 bottle (80). Phosphate-buffered detergent solution. Preservative: 0.01% thimerosal.

(7) Positive control (Human)

1 vial (0.3 mL). Inactivated human serum or plasma containing antibodies to HCV (anti-HCV) and non-reactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). Preservatives: 0.1% sodium azide and 0.05% gentamicin sulfate.

(8) Negative control (Human)

1 vial (0.3 mL). Human serum or plasma nonreactive for HBsAg, antibody to HIV-1, antibody to HIV-2 and anti-HCV. Preservatives: 0.1% sodium azide and 0.05% gentamicin sulfate.

D. Reagent Preparation

Bring the reagents to room temperature (15-30°C) and mix thoroughly by gently inverting the container several times.

Avoid foaming.

## 7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve

No calibration curve is generated by the user as part of these assay methods. The calibration of instruments is either automatic or performed periodically by contracted service personnel.

B. Verification

Not Applicable

## 8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

#### A. Preliminaries

- (1) The maximum run size is 30 strips (including a Positive and Negative control strip).
- (2) The minimum run size is 3 strips (including a Positive and Negative control strip).
- (3) Each run must proceed to completion without interruption.
- (4) The positive and negative controls should be assayed with each series of patient/donor specimens. The positive and negative assays should be treated exactly as patient/donor specimens throughout the assay procedure.
- (5) An adequate amount of Working Wash Buffer and Working Substrate should be prepared before using the reagents.
- (6) If during the course of the assay it is determined that not enough Working Wash Buffer or Working Substrate has been prepared to complete the assay procedure, the assay run is considered invalid and must be repeated using a single preparation of the Working Solution.
- (7) Fading of the strips is prevented by keeping the developed strips out of strong light (direct sunlight) and away from heat (greater than 30°C).

#### B. Sample Preparation

- (1) Bring serum specimens to 20-25°C. 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 4-8°C for extended periods. Mix specimens gently before testing.
- (3) Identify the reaction tray wells for each specimen or control.

#### C. Instrument Setup

- (1) Turn on the instrument using the power switch located at the right rear of the instrument. The startup screen will be displayed and indicate that the instrument is proceeding with the system check. Within 5 minutes the main menu should be displayed indicating that the instrument has completed initialization.
- (2) From the Main Menu select Run Assay. The following steps will then need to be performed:
  - a) Prep: Select the assay to be run and the number of specimens and enter the kit lot and operator information.
  - b) Load Reagents: Load and verify the quantities of reagents loaded. The instrument will provide instructions on volume of reagents needed for the samples to be tested.
  - c) Load Reaction Vessels: Load reaction vessels (RVs) onto the carousel

d) Begin Run: Start the assay process.

D. Operation of Assay Procedure

The instrument performs the assay procedure.

E. Recording of Data

A NEGATIVE, INDETERMINATE, or POSITIVE interpretation is based on the reaction pattern present on the strip. For valid runs the following criteria should be used for interpretation:

**Antigen Band Pattern Interpretation**

No HCV bands present having 1+ or greater reactivity Or hSOD band alone having 1+ or greater reactivity	NEGATIVE
Any single HCV band having 1+ or greater reactivity Or hSOD band having 1+ or greater reactivity in conjunction with one or more HCV bands having 1+ or greater reactivity	INDETERMINATE
At least two HCV bands having 1+ or greater reactivity	POSITIVE

A band intensity less than the IgG Control Level I (i.e., +/-) is below the cutoff for reactivity in the assay.

A POSITIVE strip interpretation can only be made in the absence of reactivity to the hSOD band (i.e., - or +/-).

A POSITIVE test results 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 INDETERMINATE be retested after six months using a freshly drawn specimen. A NEGATIVE test result by CHIRON RIBA 3.0 SIA 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.

On rare occasions, a strip may have a dark background. If the Level I IgG and Level II IgG internal control bands are indistinguishable from the background (i.e., darker than the background, with the Level II IgG control darker than the Level I IgG control), the strip is interpretable and the intensity of the bands should be compared to the internal controls as described above. In anti-HCV negative specimens or specimens lacking antibodies to one or

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more antigens present on the strip, the antigen bands may appear lighter than the back ground of the strip. Such bands should be interpreted a nonreactive (i.e., - or +/-).

F. Replacement and Periodic Maintenance of Key Components

(1) Replacement:

When the internal printer is out of paper, the Check Printer pop-up screen or Printer Error screen displays, prompting the user to check the printer paper supply and replenish if necessary.

(2) Maintenance:

Periodic maintenance of the RIBA Processor System involves both end-of-run maintenance procedures as well as monthly maintenance procedures. Both should be recorded in Maintenance Log.

End-of-Run Maintenance:

- Clean the instrument
- Dispose of the reagent container fluids
- Clean reagent containers
- Dispose of reaction vessels
- Check waste container – empty if necessary

Monthly Maintenance:

- Clean reaction chamber bowl
- Clean detection windows
- Clean air filter
- Clean outside of probe

G. Calculations

The instrument performs any calculations that are needed.

H. Special Procedure Notes

None.

## 9. REPORTABLE RANGE OF RESULTS

Anti-HCV reactivity in a specimen is determined by comparing the intensity of each HCV band to the intensity of the human IgG (Level I and Level II) internal control bands on each strip. The identity of the antibodies is defined by the specified location of the HCV band as shown in Quality Control Procedures.

The intensity of the HCV bands is scored in relation to the intensity of the internal IgG controls as follows:

	<b>Intensity of Band</b>	<b>Score</b>
Absent		-
Less than intensity of the Level I IgG control band		+/-
Equal to intensity of the Level I IgG control band		1+
Greater than the intensity of the Level I IgG control band and less than the intensity of the Level II IgG control band		2+
Equal to intensity of the Level II IgG control band		3+
Greater than intensity of the Level II IgG control band		4+

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

## 10. QUALITY CONTROL (QC) PROCEDURES

A. The assay controls supplied with the test kit must be included with each run, regardless of the number of specimens tested or strips used.

B. The identity and location of the antigens coated on the strips are in the order below from the top of the strip (strip number) to the bottom. • Strip number

- IgG Control Level II
- c-100(p);5-1-5(p)
- c33c
- c22(p)
- NS5
- hSOD
- IgG Control Level I

C. Two levels of human IgG (Level I, low control; and Level II, high control) are included on each strip as internal controls. The reactivity of the individual HCV bands is determined by comparing the intensity of each band to the Level I and Level II human IgG internal strip controls as described in Recording of Data.

D. The following results are expected from the Positive and Negative Controls supplied with the test kit:

- (1) The internal IgG control Level I and control Level II on the Positive Control, Negative Control and each test specimen must be clearly distinguishable by eye, and the IgG control Level II must be visibly lighter than the IgG control Level I.
- (2) The Positive Control strip must show a response of 2+ or greater for all HCV bands. Response to the hSOD band must be visibly lower than the Level I human IgG control (i.e., - or +/-).
- (3) The Negative Control strip must show a response to each of the HCV and hSOD bands, which is visibly lower than the Level I, human IgG control (i.e., - or +/-).

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

Additionally, the IgG control Level I and control Level II bands must be clearly distinguishable by eye on all patient/donor specimen strips, and the IgG control Level I must be clearly lighter than the IgG control Level II. If these criteria are not met for an individual patient/donor specimen, the assay must be repeated for that specimen.

*Note: If incomplete banding, or any such artifact, on a patient/donor specimen strip hinders interpretation, but the kit Positive and Negative control strips are interpretable, the patient/donor specimen strip is invalid and the assay must be repeated for that specimen.*

## **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. Closely monitor this procedure and the interpretation of results when testing serum or plasma specimens for the presence of antibody to HCV. Do not use heat-activated specimens. 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 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. Specimens may contain antibodies to either vector proteins or fusion proteins associated with the HCV recombinant antigens. Vector and/or fusion protein antibody-containing specimens may demonstrate reactivity that is unrelated to HCV infection. Additional, more specific, tests may be useful in defining the true HCV antibody reactivity.

## **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 20-25°C during preparation and testing for up to 4 hours.

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

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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.

## **REFERENCES**

Ortho Clinical Diagnostics Inc., Johnson & Johnson. Strip Immunoblot Assay (SIA) for the Detection of Antibodies to Hepatitis C Virus (Anti-HCV) in Human Serum or Plasma – CHIRON RIBA HCV 3.0 Strip Immunoblot Assay (SIA) directional literature included with each assay kit: Chiron Corporation, Emeryville, CA,2001.

U.S. Department of Health and Human Services. Biosafety in microbiological and biomedical laboratories. HHS Publication (NIH) 93-8395. Washington:U.S. Government Printing Office, 1993.

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