

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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Revised: N/A

As performed by: Assay Development and Diagnostic Reference Laboratory (ADDRL)
Laboratory Branch
Division of Viral Hepatitis
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

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

Important Information for Users

The National Center for HIV/AIDS, 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.

February 24, 2011

Public Release Data Set Information

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

| Data File Name | Variable Name | SAS Label |
|----------------|---------------|----------------------------------|
| HEPC_F | LBDHCV | Hepatitis C antibody (confirmed) |

February 24, 2011



Intended for Use in the United States

INSTRUCTIONS FOR USE aHCV

VITROS Immunodiagnostic Products

Anti-HCV Reagent Pack

REF 680 1325

Version 4.2 Pub. No. J03805_EN

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

Intended Use

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 System.

Three recombinant hepatitis C virus encoded antigens are used.

Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with hepatitis C virus, (state or associated disease not determined), in persons with signs or symptoms of hepatitis and in persons at risk for hepatitis C infection. In addition, this assay may be used to screen for Hepatitis C Infection in pregnant women to identify neonates who are at high risk of acquiring HCV during the prenatal period.

WARNING: *This assay has not been FDA cleared or approved for the screening of blood or plasma donors.*

Assay performance characteristics have not been established for testing a pediatric population less than 10 years of age.

Summary and Explanation of the Assay

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. ¹

Three recombinant hepatitis C virus encoded antigens (c22-3, c200 and NS5) are used in the VITROS Anti-HCV assay. 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.² 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. ³

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

Principles of the Procedure

The VITROS Anti-HCV assay is performed using the VITROS Anti-HCV Reagent Pack and VITROS Immunodiagnostic Products Anti-HCV Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System (VITROS Immunodiagnostic 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.

A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. ⁴ The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent increases the level and duration of the light produced. The light signals are read by the VITROS Immunodiagnostic System. The amount of HRP conjugate bound is indicative of the level of anti-HCV present in the sample.

Assay Type

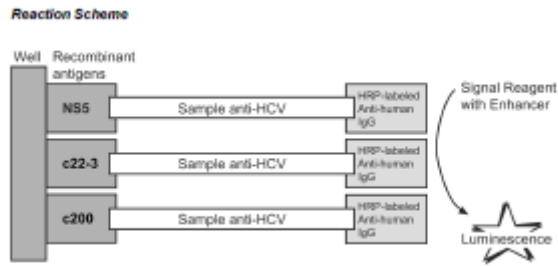
Immunometric assay

Assay Time and Temperature

Incubation time: 45 minutes

February 24, 2011

Time to first result: 55 minutes
 Temperature: 37 °C



Warnings and Precautions

For *in vitro* diagnostic use only.

- Treat as if capable of transmitting infection.
- Handling of samples and assay components, their use, storage and solid and liquid waste disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation (e.g. NCCLS Guideline M29). ^{5, 6}

The VITROS Anti-HCV Calibrator is the only component that contains human-derived material. The calibrator contains:

- HCV antibody positive plasma obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen, and for antibodies to the human immunodeficiency virus (HIV 1+2), using FDA approved methods (enzyme immunoassays). The HCV antibody positive plasma has been treated in order to reduce the titer of potentially infectious virus. However, as no testing method can rule out the risk of potential infection, handle as if capable of transmitting infection.

- HCV antibody negative plasma obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen, and for antibodies to HCV and HIV 1+2, using FDA approved methods (enzyme immunoassays). Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent.

WARNING: The conjugate reagent in the VITROS Anti-HCV Reagent Pack contains ProClin 300 (1% w/w). The total ProClin 300 content is 206 mg*.

The VITROS Anti-HCV Calibrator contains Kathon (2% w/w). The total Kathon content in the calibrator is 40 mg.

Hazard warnings for the components containing ProClin300 and Kathon:

R43 - May cause sensitization by skin contact.

S24 - Avoid contact with skin

S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection.

Reagents

Reagent Pack Contents

One VITROS Anti-HCV Reagent Pack, 100 tests contains:

- 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 buffered fetal calf serum with anti-microbial agent (1% ProClin 300 w/w).

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- Reagent packs do not need mixing.
- Avoid agitation, which may cause foaming or the formation of bubbles.

February 24, 2011

Reagent Pack Stability

When stored and handled as specified in the package labeling, the VITROS Anti-HCV Reagent Pack is suitable for use until the expiration date printed on the outside of the carton.

Reagent Pack Storage and Preparation

- Store the unopened reagent pack refrigerated at 2-8 °C (36-46 °F). Do not freeze.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Use opened reagent packs within 8 weeks.
- Store opened reagent packs in the VITROS Immunodiagnostic System reagent supply, or refrigerated at 2-8 °C (36-46°F) in a sealed reagent pack storage box that contains dry desiccant.

Specimen Collection and Preparation

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum, EDTA, heparin or citrated plasma.

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.

Specimens Not Recommended

Turbidity in samples may affect assay results.

Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies. ⁸ Because of the variety of sample collection devices available, it is not possible to issue a definitive statement on the performance of VITROS Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer's instructions and is compatible with this assay.

Specimen Collection and Preparation

- Collect specimens using standard procedures. ⁹
- The VITROS Anti-HCV assay uses 20 μ L of sample for each determination.
- For details on minimum fill volume of sample cups or containers, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.
- Mix samples, calibrator, and controls by inversion and bring to 15-30 °C (59-86 °F) before use.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.

Handling and Storage Conditions

- Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.
- The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for further information.
- The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing specimens: ¹⁰
 - Store samples at 22 °C (72 °F) for no longer than 8 hours.
 - If the assay will not be completed within 8 hours, refrigerate samples at 2-8 °C (36-46 °F).
 - If the assay 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.

Assay Procedure

February 24, 2011

Materials Required But Not Provided

The following items are required to perform the VITROS Anti-HCV assay:

- VITROS ECi/ECiQ Immunodiagnostic System
- VITROS Anti-HCV Calibrator
- 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

Operating Instructions

Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for complete instructions on the operation of your VITROS Immunodiagnostic System.

Calibration

Required Calibrator

VITROS Anti-HCV Calibrator

Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of the VITROS Anti-HCV Calibrator.

Calibration Procedure

- Calibration must be performed using a calibrator of the same lot number as the reagent pack.
- Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for detailed instructions on how to calibrate.

When to Calibrate

- Calibrate when the lot of reagent pack and calibrator changes
- Calibrate every 28 days

The VITROS Anti-HCV assay may also need to be recalibrated:

- After specified service procedures have been performed (see the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide)
- If quality control results are consistently outside of the manufacturer's or your acceptable range.

For additional information on when to calibrate, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.

Quality Control

Procedure Recommendations

- Choose control levels that check performance at clinically relevant points. The recommendation is to run a negative control and a positive control close to the anti-HCV decision point (signal/cutoff [s/c] ≥ 1.00).
- To verify system performance, analyze control materials:
 - After calibration
 - At least once every 24 hours
 - After specified service procedures or maintenance to critical parts or subsystems that might influence performance of the assay (see the VITROS ECi/ECiQ immunodiagnostic System Operator's Guide)
- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
- For more detailed information on quality control procedures, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.

February 24, 2011

- Refer to Internal Quality Control Testing: Principles and Definitions or other published guidelines for general quality control recommendations. ¹¹
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Quality Control Material Selection

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed. ¹² VITROS Anti-HCV Controls are recommended for use with the VITROS Immunodiagnostic System. The performance of other commercial control fluids should be evaluated for compatibility with this assay before they are used for quality control. Appropriate quality control value ranges must be established for all commercially available quality control materials used with the VITROS Anti-HCV assay.

Quality Control Material Preparation and Storage

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

Interpretation of Results and Expected Results

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

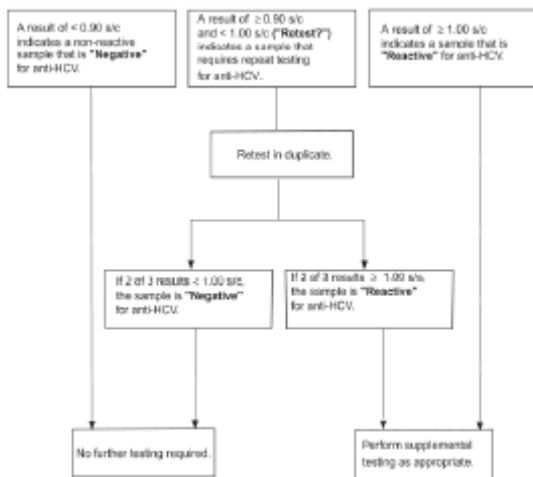
$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

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.

| 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



Interpretation of Results

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

February 24, 2011

| Final VITROS Anti-HCV Assay 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 1996;47 (RR-15)

- Results obtained with the VITROS Anti-HCV assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- The magnitude of a VITROS Anti-HCV assay 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.

Expected Results

Approximately 65.8% (1724/2622) of the study subjects participating in the VITROS Anti-HCV clinical study reported no recent or current signs or symptoms of hepatitis. Of the 1724 asymptomatic individuals, 26.3% were enrolled in Miami, FL, 36.4% were enrolled in Dallas, TX, 25.1% were enrolled in Chicago, IL, and 12.2% were enrolled in Los Angeles, CA. The group was Caucasian (28.8%), African American (41.0%), Hispanic (21.9%), and Asian (3.8%), with the remaining 4.6% represented by other ethnic groups. The group was 56% male and 44% female and ranged in age from two to 96 years. All were at risk for hepatitis or HCV infection due to lifestyle, behavior, occupation or known exposure event or belonged to clinical groups at risk for HCV infection. The VITROS Anti-HCV assay was reactive in 23.9% of the individuals in this group. The percent VITROS Anti-HCV reactive results observed in the asymptomatic population at each site was 28.0% at Miami, FL, 27.9% at Dallas, TX, 14.8% at Chicago, IL, and 21.8% at Los Angeles, CA.

The table below summarizes the distribution of VITROS Anti-HCV reactive and negative results among the study subjects without signs or symptoms of hepatitis, by age and gender.

| Age Range | Gender | VITROS Anti-HCV Result | | | | Total |
|--------------|--------|------------------------|-------------|-------------|-------------|--------------|
| | | Reactive | | Negative | | |
| | | N | Percent | N | Percent | |
| 0-9 | F | 0 | 0 | 0 | 0 | 0 |
| | M | 11 | 78.6 | 3 | 21.4 | 14 |
| 10-19 | F | 0 | 0.0 | 21 | 100 | 21 |
| | M | 22 | 68.8 | 10 | 31.3 | 32 |
| 20-29 | F | 7 | 5.4 | 122 | 94.6 | 129 |
| | M | 30 | 24.4 | 95 | 75.6 | 125 |
| 30-39 | F | 20 | 12.7 | 137 | 87.3 | 157 |
| | M | 78 | 30.0 | 182 | 70.0 | 260 |
| 40-49 | F | 37 | 21.4 | 136 | 78.6 | 173 |
| | M | 111 | 39.8 | 168 | 60.2 | 279 |
| 50-59 | F | 20 | 15.0 | 113 | 85.0 | 133 |
| | M | 41 | 29.5 | 98 | 70.5 | 139 |
| 60-69 | F | 11 | 12.4 | 78 | 87.6 | 89 |
| | M | 14 | 20.0 | 56 | 80.0 | 70 |
| 70-79 | F | 3 | 6.7 | 42 | 93.3 | 45 |
| | M | 6 | 15.4 | 33 | 84.6 | 39 |
| 80-89 | F | 0 | 0.0 | 10 | 100 | 10 |
| | M | 0 | 0.0 | 8 | 100 | 8 |
| 90-100 | F | 0 | 0 | 0 | 0 | 0 |
| | M | 1 | 50.0 | 1 | 50.0 | 2 |
| Total | | 412 | 23.9 | 1309 | 78.1 | 1721* |

* Age was not reported for three subjects.

Limitations of the Procedure

- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with HCV. HCV antibodies may be undetectable in some stages of the infection and in some clinical conditions. ¹³ Follow CDC recommendations for supplemental testing of reactive samples.¹⁴
- Results from immunosuppressed individuals should be interpreted with caution.
- The prevalence of the analyte will affect the assay's predictive value.
- Assay performance characteristics have not been established for any other specimens 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. ¹⁵ 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 assay with other flaviviruses known to cause hepatic disease has not been

February 24, 2011

established.

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

Performance Characteristics

Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the VITROS Anti-HCV assay among individuals with specific risks or history associated with HCV infection, including transfusions or transplants before 1992, past and current use of intravenous drugs (IVDU), chronic (long term) hemodialysis, and hemophiliacs who had received clotting factors produced prior to 1987. Also included were individuals with signs or symptoms or biochemical manifestations (elevated liver function tests) of hepatitis and those at high risk of hepatitis infection due to lifestyle, behavior, occupation, clinical condition, or known exposure events. Specimens were obtained from 2644 subjects prospectively enrolled at five geographically separated collection sites within the United States located in Miami, FL (35.8%), Dallas, TX (28.5%), Chicago, IL (22.7%), and Los Angeles, CA (13.0%). Of these, 2622 were available for testing and analysis. Statistical testing was performed to ensure that the distribution of VITROS Anti-HCV s/c values were homogeneous across the five collection sites, indicating that the data could be pooled for analysis.

The group was Caucasian (26.3%), African American (39.1%), and Hispanic (26.7%), with the remaining 8.0% represented by other ethnic groups. The group was 54.3% male and 45.7% female, and ranged in age from two to 96 years. The HCV status for each subject was determined from the results of a reference assay for the detection of anti-HCV and *Chiron* RIBA* HCV 3.0 SIA*, when required. In addition, reference assays for HBsAg, HBsAg Confirmatory, and anti-HAV IgM were performed to determine co-infection with HBV or HAV, respectively. All reference testing during the clinical laboratory study was performed following manufacturer's instructions using assays previously licensed or approved by the FDA. VITROS Anti-HCV testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (35.9%), Los Angeles, CA (35.8%), and Minneapolis, MN (28.3%).

Results by Specimen Classification

Following testing with the reference anti-HCV assay and supplemental testing with the *Chiron* RIBA* HCV 3.0 SIA* where indicated, 2607 subjects were assigned an HCV status of HCV infected or not HCV infected based on the final results obtained with both assays as required. The HCV status of the remaining 15 subjects could not be determined due to indeterminate results with the *Chiron* RIBA* HCV 3.0 SIA*. Assignment of HCV status is presented in the table below.

| Reference Anti-HCV Assay Result | Chiron* RIBA* HCV 3.0 SIA Result | HCV Status |
|---------------------------------|----------------------------------|--|
| Negative | Not Applicable | Not HCV Infected |
| Repeatedly Reactive | Positive | HCV Infected (State or Associated Disease Not Determined) |
| Repeatedly Reactive | Negative | Not HCV Infected |
| Repeatedly Reactive | Indeterminate | Not Determined (HCV Status Cannot be Determined) |

* Trademark

Comparison of Results

The table below compares VITROS Anti-HCV assay results with HCV status according to a ranking of the risk of HCV infection in study subjects (N=2622). The ranking was based on a clinical evaluation of the chances of acquiring the disease through the following modes of transmission, with the most common given higher rankings. Each patient was assigned only one risk (the highest).¹⁴ Assignment of HCV status was according to the algorithm presented in the previous table.

| Hepatitis Ranked Risk Group (Transmission Mode) | HCV Status | | | | | | Total |
|---|------------------------|----------|------------------------|----------|------------------------|----------|-------|
| | HCV Infected | | Not Determined | | Not HCV Infected | | |
| | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | |
| | Negative | Reactive | Negative | Reactive | Negative | Reactive | |
| Hemophiliac | 0 | 67 | 0 | 0 | 6 | 1 | 94 |
| IVDU, Current or Past | 0 | 247 | 0 | 5 | 64 | 4 | 340 |
| Dialysis Patients | 0 | 59 | 1 | 4 | 317 | 7 | 388 |
| Transfusion/Transplant | 0 | 84 | 0 | 2 | 274 | 3 | 363 |
| High Risk Sex | 0 | 55 | 1 | 1 | 448 | 7 | 512 |
| Healthcare Worker | 0 | 12 | 0 | 1 | 210 | 2 | 225 |
| Others/Unknown | 0 | 68 | 0 | 0 | 384 | 3 | 455 |
| None Specified | 1 | 36 | 0 | 0 | 207 | 1 | 245 |
| Overall | 1* | 648 | 2 | 13 | 1930 | 28** | 2622 |

* HCV RNA was not detected by the COBAS AMPLICOR[®] Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems, Inc.).

** Three of the 28 samples were repeatedly reactive with the reference anti-HCV assay and negative with the *Chiron* RIBA* HCV 3.0 SIA*. No additional supplemental PCR testing was performed. The hemophiliac sample was negative with the reference anti-HCV assay, and had insufficient volume for supplemental SIA or PCR testing. The remaining 24 samples were tested with the *Chiron* RIBA* HCV 3.0 SIA* and the AMPLICOR[®] Hepatitis C Virus (HCV) Test, version 2.0 (Roche Molecular Systems, Inc.). Three samples were positive with *Chiron* RIBA* HCV 3.0 SIA* and five samples had HCV RNA detected by PCR supplemental testing. Thus eight of 28 VITROS Anti-HCV assay presumably false positive samples had evidence of active or past HCV infection. These results were not applied to the calculation of percent agreement.

February 24, 2011

The HCV status of 15 subjects could not be determined following testing with the reference anti-HCV assay (all were repeatedly reactive) and the *Chiron* RIBA* HCV 3.0 SIA* (all had indeterminate results). Additional supplemental testing for HCV RNA by PCR was performed on the 15 samples using the COBAS AMPLICO™ Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems, Inc.). The results of this testing and the HCV status of the 15 samples following supplemental PCR testing are presented in the following table.

| VITROS Anti-HCV Assay Result | HCV RNA by PCR | HCV Status Following Supplemental Testing | Number of Samples | Hepatitis Ranked Risk Group |
|------------------------------|----------------|---|-------------------|-----------------------------|
| Reactive* | Detected** | HCV Infected | 3 | IVDU, Current or Past |
| | | | 2 | Dialysis Patients |
| | | | 1 | Transfusion/Transplant |
| Reactive*** | Not Detected | Not Determined | 2 | IVDU, Current or Past |
| | | | 1 | Transfusion/Transplant |
| | | | 1 | High Risk Sex |
| | | | 1 | Healthcare Worker |
| | | | 2 | Dialysis Patient |
| Negative**** | Not Detected | Not Determined | 1 | High Risk Sex |
| | | | 1 | Dialysis Patients |
| Total | | | 15 | |

* A laboratory diagnosis of 'HCV Infected' was made following supplemental PCR testing (SIA indeterminate/HCV RNA detected by PCR). The VITROS Anti-HCV result is presumed to be correct (true positive).

** Indicates active HCV infection.

*** An accurate laboratory determination of HCV status could not be made following supplemental PCR testing (SIA indeterminate/HCV RNA not detected by PCR). The VITROS Anti-HCV result is presumed to be incorrect (false positive).

**** An accurate laboratory determination of HCV status could not be made following supplemental PCR testing (SIA indeterminate/HCV RNA not detected by PCR). The VITROS Anti-HCV result is presumed to be incorrect (false negative).

Percent Agreement

Percent positive and percent negative agreement between the VITROS Anti-HCV assay and HCV status were calculated for subjects with various risks for viral hepatitis or HCV infection, and for the overall study population (N=2622). The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals. For purposes of calculating percent agreement, VITROS Anti-HCV assay reactive samples whose HCV status remained 'Not Determined' following supplemental PCR testing were considered 'Not HCV Infected' (false positive - seven samples), and VITROS Anti-HCV assay negative samples whose HCV status remained 'Not Determined' following PCR testing were considered 'HCV Infected' (false negative - two samples).

| Hepatitis Ranked Risk Group | Positive Percent Agreement | 95% Exact Confidence Interval | Negative Percent Agreement | 95% Exact Confidence Interval |
|-----------------------------|----------------------------|-------------------------------|----------------------------|-------------------------------|
| Overall | 99.54 (654/657) | 98.67-99.91 | 98.22 (1930/1965) | 97.53-98.76 |
| Hemophilic* | 100.0 (87/87) | 95.85-100.0 | 85.71 (6/7) | 42.13-99.64 |
| IVDU, Current or Past | 100.0 (250/250) | 98.54-100.0 | 93.33 (84/90) | 88.05-97.51 |
| Dialysis Patients | 98.39 (61/62) | 91.34-99.96 | 97.24 (317/326) | 94.80-98.73 |
| Transfusion/Transplant | 100.0 (85/85) | 95.75-100.0 | 98.56 (274/278) | 96.36-99.61 |
| High Risk Sex | 98.21 (55/56) | 90.45-99.95 | 98.25 (448/456) | 96.57-99.24 |
| Healthcare Worker | 100.0 (12/12) | 73.54-100.0 | 98.59 (219/219) | 95.94-99.71 |
| Others/Unknown | 100.0 (88/88) | 94.72-100.0 | 99.22 (384/387) | 97.75-99.64 |
| None Specified | 97.30 (38/37) | 85.84-99.95 | 99.52 (207/208) | 97.35-99.99 |

* Includes 16 individuals under 10 years of age.

The percent positive agreement with HCV status was determined by dividing the number of reactive VITROS Anti-HCV results by the total number of subjects determined to be 'HCV infected'. As a result of this study, the overall positive percent agreement of the VITROS Anti-HCV assay with HCV status was estimated to be 99.54% (654/657, with a 95% exact confidence interval of 98.67% to 99.91%). There were no differences in positive percent agreement among subjects in the various ranked risk groups.

The percent negative agreement with HCV status was determined by dividing the number of negative VITROS Anti-HCV assay results by the number of subjects determined to be 'Not HCV Infected'. As a result of this study, the overall negative percent agreement of the VITROS Anti-HCV assay with HCV status was estimated to be 98.22% (1930/1965, with a 95% exact confidence interval of 97.53% to 98.76%). There were no differences in negative percent agreement among subjects in the various ranked risk groups.

Clinical Performance in Pregnant Women

Prospectively collected serum samples from 547 pregnant women in several different locations in the US who were at low risk or high risk for exposure to HCV were tested to assess the clinical performance of the VITROS Anti-HCV assay in testing for hepatitis C virus infection during all three trimesters of pregnancy. Of the 547 prospectively collected samples, 64.2% were obtained in Florida, 27.2% in California, 6.8% in Texas and 1.8% in Connecticut. Of the combined population, 45.5% were obtained during the first trimester, 24.1% during the second trimester and 30.3% during the third trimester. The following table furnishes a breakdown of the 547 subjects in the prospective population. The table does not include an additional 50 subjects (from Indiana) from whom unlinked residual laboratory samples were randomly acquired during labor and delivery or the

February 24, 2011

postpartum period, since demographic information was not available for those subjects.

| Demographic Profiles of Pregnant Women at Low or High Risk for Hepatitis (Excluding Labor and delivery) | | | |
|---|----------------------|-------------|--------------|
| Demographic Profile | Viral Hepatitis Risk | | Total N (%)* |
| | Low N (%)* | High N (%)* | |
| TOTAL | 297 (54.3%) | 250 (45.7%) | 547 (100.0%) |
| TRIMESTER | | | |
| First | 95 (17.4%) | 154 (28.2%) | 249 (45.5%) |
| Second | 84 (15.4%) | 48 (8.8%) | 132 (24.1%) |
| Third | 118 (21.6%) | 48 (8.8%) | 166 (30.5%) |
| ETHNICITY | | | |
| African-American | 22 (4.0%) | 31 (5.7%) | 53 (9.7%) |
| Asian/Pacific Islander | 58 (10.2%) | 4 (0.7%) | 62 (11.2%) |
| Caucasian | 147 (26.9%) | 98 (18.0%) | 145 (26.5%) |
| Hispanic | 4 (0.7%) | 6 (1.1%) | 10 (1.8%) |
| Other | 88 (12.1%) | 155 (30.2%) | 231 (42.2%) |
| Unknown | 2 (0.4%) | 2 (0.4%) | 4 (0.7%) |
| Unknown | 0 (0%) | 6 (1.1%) | 6 (1.1%) |
| AGE | | | |
| Age 17-30 | 145 (26.5%) | 177 (32.4%) | 322 (58.9%) |
| Age 31-48 | 151 (27.6%) | 73 (13.3%) | 224 (41.0%) |
| Age Unknown | 1 (0.2%) | 0 (0%) | 1 (0.2%) |

*The number (N) of subjects; Expressed as a percentage (%) of analyzed subjects (N=547).

Agreement of the VITROS Anti-HCV assay was assessed relative to HCV status using serum samples obtained from a total of 547 women at high risk or low risk for hepatitis. The tables below compare the VITROS Anti-HCV assay results with HCV status among the overall population of pregnant women by risk and trimester.

| VITROS Anti-HCV Assay Results and HCV Status among Pregnant Women at High Risk for Hepatitis | | | | | | | | | | | | |
|--|-----------------|-----|----|-------|------------------|----|----|-------|-----------------|----|----|-------|
| VITROS Anti-HCV Result | First Trimester | | | | Second Trimester | | | | Third Trimester | | | |
| | HCV Status | | | Total | HCV Status | | | Total | HCV Status | | | Total |
| | + | - | ND | | + | - | ND | | + | - | ND | |
| Reactive | 3 | 1 | 1 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 |
| Negative | 0 | 149 | 0 | 149 | 0 | 48 | 0 | 48 | 0 | 46 | 0 | 46 |
| Total | 3 | 150 | 1 | 154 | 0 | 48 | 0 | 48 | 1 | 46 | 1 | 48 |

| VITROS Anti-HCV Assay Results and HCV Status among Pregnant Women at Low Risk for Hepatitis | | | | | | | | | | | | |
|---|-----------------|----|----|-------|------------------|----|----|-------|-----------------|-----|----|-------|
| VITROS Anti-HCV Result | First Trimester | | | | Second Trimester | | | | Third Trimester | | | |
| | HCV Status | | | Total | HCV Status | | | Total | HCV Status | | | Total |
| | + | - | ND | | + | - | ND | | + | - | ND | |
| Reactive | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 2 |
| Negative | 0 | 95 | 0 | 95 | 0 | 83 | 0 | 83 | 0 | 118 | 0 | 118 |
| Total | 0 | 95 | 0 | 95 | 1 | 83 | 0 | 84 | 0 | 118 | 0 | 118 |

The table below summarizes the percent agreement between the VITROS Anti-HCV assay and HCV status for this population. The table provides the 95% exact confidence intervals.

| Positive and Negative Percent Agreement of the VITROS Anti-HCV Assay with HCV Status in Pregnant Women | | | | |
|--|----------------------------|-------------------------------|----------------------------|-------------------------------|
| Subjects | Positive Percent Agreement | 95% Exact Confidence Interval | Negative Percent Agreement | 95% Exact Confidence Interval |
| Pregnant Women | 100.0 (5/5) | 47.8-100.0 | 99.08 (537/542) | 97.8-99.7 |

50 residual laboratory samples obtained during postpartum hospital stay were tested with the VITROS Anti-HCV assay and a reference assay. All 50 samples were found to be negative for anti-HCV. Negative percent agreement = 100% (50/50) (95% exact confidence interval 92.9-100.0).

Seroconversion Panels

Twenty commercially available seroconversion panels were tested. The VITROS and reference anti-HCV assay results are summarized below. The table lists the first bleed of each panel that tested reactive with the VITROS and the reference assays as well as the difference between the two assays in identifying the first reactive panel member by number of days.

February 24, 2011

| Panel ID | Days to Evidence of HCV Infection | | | | | | | | | | Difference in Days to Anti-HCV Reactive Result Reference - VITROS |
|----------|-----------------------------------|------|-----------------------|-----|----------|-----|------------------------|-----|-----------|----|--|
| | Reference Anti-HCV Assay | | VITROS Anti-HCV Assay | | HCV RNA* | | Supplemental Testing** | | IND ***** | | |
| | - | **** | - | + | - | + | - | + | - | + | |
| 8211 | 186 | 189 | 171 | 182 | 121 | 140 | 171 | 182 | 186 | | 7 |
| 8212 | 37 | 53 | 0 | 12 | | | 0 | 14 | 23 | | 41 |
| 8213 | 37 | 43 | 35 | 37 | 8 | 11 | 37 | | 43 | | 6 |
| 8214 | 32 | 49 | 25 | 30 | | | 0 | 25 | 30 | 49 | 19 |
| 8215 | 20 | | 20 | | | | 0 | 10 | 20 | | N/A |
| 8216 | 17 | 23 | 17 | 23 | 17 | 23 | 17 | 23 | | | 0 |
| 8222 | 40 | | 38 | 40 | 2 | 17 | 38 | 40 | | | >0 |
| 8224 | 22 | | 11 | 19 | | | 0 | 11 | 19 | | >3 |
| 8225 | 80 | | 73 | 78 | 39 | 45 | 73 | 78 | | | >2 |
| 8227 | 48 | 74 | 48 | 74 | 24 | 42 | 48 | | 74 | | 0 |
| 8228 | 38 | | 24 | 28 | | | 0 | 28 | 31 | | >10 |
| 8229 | 24 | 28 | 17 | 20 | | | 0 | 20 | 24 | | 8 |
| PHV905 | 21 | 25 | 14 | 18 | | | 0 | 7 | 11 | 21 | 7 |
| PHV906 | 14 | 17 | | 0 | | | 0 | 0 | 7 | | 17 |
| PHV907 | 13 | 18 | 7 | 13 | | | 0 | 7 | 13 | 21 | 5 |
| PHV908 | 45 | 48 | 19 | 25 | | | 0 | 11 | | 13 | 23 |
| PHV909 | 0 | 28 | 0 | 28 | | | 0 | 0 | 28 | | 0 |
| PHV911 | 3 | 14 | 3 | 14 | | | 0 | 3 | | 14 | 0 |
| PHV912 | 4 | 7 | 3 | 7 | | | 0 | 4 | 7 | | 0 |
| SC-0100 | 0 | 7 | | 0 | | | | 0 | | | 7 |

* Research assay not verified for clinical use.
 ** Chiron* RIBA* HCV 3.0 SIA
 **** Post bleed day of last nonreactive result, usually denotes previous bleed from first reactive.
 ***** Post bleed day of first reactive result.
 ***** Post bleed day of last nonreactive result, usually denotes previous bleed from first reactive.

Genotype Detection

Genotype detection was assessed using the Boston Biomedica, Inc. Worldwide HCV Performance Panel. The panel consisted of 20 human plasma samples that were predetermined by the supplier to include four of the six recognized genotypes of HCV and their most common subtypes (1a, 1b, 1a/b, 2a/c, 3a/b, 4c/d, 4h). All of the anti-HCV positive panel members (18/18) were observed to be reactive in the VITROS Anti-HCV assay and the two anti-HCV negative control panel members were negative by the VITROS Anti-HCV assay. In additional studies, 7/7 samples characterized to be genotype 5a by the supplier tested VITROS Anti-HCV reactive, while 1/1 sample characterized as genotype 6 was VITROS Anti-HCV reactive.

Potentially Cross-Reacting Subgroups

Samples with evidence of Hepatitis B infection (HBV) or Hepatitis A infection (HAV) were identified in a population of 2622 prospectively collected samples. The tables below compare VITROS Anti-HCV results with HCV status according to a ranking of the risk of HCV infection in these study subjects.

Comparison of VITROS Anti-HCV Results and HCV Status Among HBsAg Positive Study subjects (N=87)

| Hepatitis Ranked Risk Group | HCV Status | | | | | | Total |
|-----------------------------|------------------------|----------|------------------------|----------|------------------------|----------|-------|
| | HCV Infected | | Not Determined | | Not HCV Infected | | |
| | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | |
| | Negative | Reactive | Negative | Reactive | Negative | Reactive | |
| IVDU, Current or Past | 0 | 8 | 0 | 1 | 1 | 0 | 8 |
| Dialysis Patient/S | 0 | 3 | 0 | 1 | 9 | 1 | 14 |
| Transfusion/Transplant | 0 | 2 | 0 | 0 | 13 | 0 | 15 |
| High Risk Sex | 0 | 1 | 0 | 0 | 16 | 0 | 17 |
| Healthcare Worker | 0 | 1 | 0 | 0 | 5 | 0 | 6 |
| All Others | 0 | 4 | 0 | 0 | 22 | 1 | 27 |
| Overall | 0 | 17 | 0 | 2 | 66 | 2 | 87 |

Comparison of VITROS Anti-HCV Results and HCV Status Among HAV-IgM Positive Study Subjects (N=9)

| Hepatitis Ranked Risk Group | HCV Status | | | | | | Total |
|-----------------------------|------------------------|----------|------------------------|----------|------------------------|----------|-------|
| | HCV Infected | | Not Determined | | Not HCV Infected | | |
| | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | VITROS Anti-HCV Result | | |
| | Negative | Reactive | Negative | Reactive | Negative | Reactive | |
| IVDU, Current or Past | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dialysis Patient/S | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Transfusion/Transplant | 0 | 0 | 0 | 0 | 4 | 0 | 4 |
| High Risk Sex | 0 | 1 | 0 | 0 | 1 | 0 | 2 |
| Healthcare Worker | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| All Others | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| Overall | 0 | 1 | 0 | 0 | 8 | 0 | 9 |

February 24, 2011

Potentially Cross-Reacting Subgroups

The specificity of the VITROS Anti-HCV assay was evaluated by testing 292 samples from 22 potentially cross-reacting sub-groups. With the exception of the Co-Infection (HBV/HCV) samples, all of the samples were previously classified as anti-HCV negative in other commercially available assays. Samples found to be ≥ 1.00 by the VITROS Anti-HCV assay were retested in duplicate. A summary of the results is given in the table below.

| Clinical Category | Number Samples Tested | VITROS Anti-HCV assay Result <1.00 | VITROS Anti-HCV assay result ≥ 1.00 |
|--|-----------------------|------------------------------------|--|
| Hepatitis A Infection (HAV) | 10 | 10 | 0 |
| Co-Infection (HBV/HCV) | 10 | 0 | 10* |
| HEV Infection (HEV) | 10 | 10 | 0 |
| Non-viral Liver Disease | 52 | 52 | 0 |
| Autoimmune Diseases (Rheumatoid Arthritis) | 50 | 50 | 0 |
| Autoimmune Diseases (Systemic Lupus Erythematosus) | 10 | 10 | 0 |
| Cytomegalovirus (CMV) | 10 | 10 | 0 |
| Epstein-Barr Virus (EBV) | 10 | 10 | 0 |
| Herpes simplex virus (HSV) | 10 | 10 | 0 |
| Parvovirus B19 Infection | 10 | 10 | 0 |
| Rubella | 10 | 10 | 0 |
| Syphilis | 10 | 10 | 0 |
| Toxoplasmosis | 10 | 10 | 0 |
| Human Immunodeficiency virus (HIV 1/2) | 11 | 11 | 0 |
| Human T-cell lymphotropic virus (HTLV 1/2) | 10 | 10 | 0 |
| Recent Influenza Vaccine Recipients | 10 | 10 | 0 |
| Heterophilic Antibodies (Human anti-mouse) | 5 | 5 | 0 |
| Yeast Infection | 10 | 10 | 0 |
| Multiparous Females | 10 | 10 | 0 |
| Multiple Transfusion Patients | 10 | 10 | 0 |
| Dialysis Patients | 10 | 10 | 0 |
| Co-Infection (HBV/HDV) | 4 | 2 | 2* |
| Total Samples Tested | 292 | 280 | 12 |

* Also reactive in the Ortho® HCV Version 3.0 ELISA Test System

Substances that do not Interfere

As recommended by NCCLS Protocol EP7¹⁵, the VITROS Anti-HCV assay was evaluated for interference by testing the substances listed in the table below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with anti-HCV to a level near the s/c of 1.00. None of the compounds at the levels tested were found to interfere with the clinical interpretation of the assay.

| Compound | Compound Concentration | |
|-------------|------------------------|------------|
| Bilirubin | 0.35 mmol/L | 20 mg/dL |
| Hemoglobin | 0.31 mmol/L | 500 mg/dL |
| Triglycerin | 33.3 mmol/L | 3000 mg/dL |

Precision

Precision was evaluated on a different VITROS Immunodiagnostic System at three external sites, using one lot of reagent. Two replicates each of a three member panel were assayed on a single occasion per day on 20 different days. The data shown in the table were rounded following all calculations.

February 24, 2011

| | Mean VITROS Anti-HCV S/C (Ratio) | Within Day* | | Between Day** | | Total*** | | No. Observ. | No. Days |
|--------|--|-------------|--------|---------------|--------|----------|--------|----------------|-------------|
| | | SD | CV (%) | SD | CV (%) | SD | CV (%) | | |
| Site 1 | 0.14 | 0.004 | 2.8 | 0.012 | 8.7 | 0.013 | 9.1 | 40 | 20 |
| | 8.44 | 0.078 | 1.2 | 0.151 | 2.3 | 0.170 | 2.8 | 40 | 20 |
| | 1.98 | 0.027 | 2.5 | 0.085 | 8.0 | 0.089 | 8.4 | 40 | 20 |
| Site 2 | 0.13 | 0.013 | 9.9 | 0.028 | 20.3 | 0.029 | 22.8 | 40 | 20 |
| | 8.35 | 0.205 | 3.2 | 0.105 | 1.7 | 0.230 | 3.6 | 40 | 20 |
| | 1.04 | 0.039 | 3.7 | 0.074 | 7.2 | 0.084 | 8.1 | 40 | 20 |
| Site 3 | 0.12 | 0.005 | 4.7 | 0.008 | 7.2 | 0.010 | 8.8 | 40 | 20 |
| | 8.85 | 0.098 | 1.5 | 0.090 | 1.5 | 0.139 | 2.1 | 40 | 20 |
| | 1.07 | 0.017 | 1.8 | 0.037 | 3.4 | 0.040 | 3.8 | 40 | 20 |

* Within Day: Variability of the assay performance from replicate to replicate.
 ** Between Day: Variability of the assay performance from day to day.
 *** Total: Variability of the assay performance combining the effects of within day and between day.

Precision was further evaluated incorporating between site and between lot variation. The study was performed at three external sites using three reagent lots. At least three replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates (CV) were derived from a variance component analysis. The data shown in the table were rounded following all calculations.

| Mean VITROS Anti- HCV S/C (Ratio) | Between Site* | | Between Lot** | | Total*** | | No. Observ. |
|--|---------------|--------|---------------|--------|----------|--------|----------------|
| | SD | CV (%) | SD | CV (%) | SD | CV (%) | |
| 0.18 | 0.008 | 4.3 | 0.031 | 17.1 | 0.034 | 18.8 | 182 |
| 0.90 | 0.000 | 0.0 | 0.000 | 0.0 | 0.055 | 6.1 | 182 |
| 1.02 | 0.017 | 1.7 | 0.000 | 0.0 | 0.078 | 7.5 | 182 |
| 4.78 | 0.088 | 1.8 | 0.270 | 5.8 | 0.333 | 7.0 | 182 |

* Between site: Variability of the assay performance from site to site.
 ** Between lot: Variability of the assay performance from lot to lot, calculated using data across all sites.
 *** Total: Total variability of the assay performance incorporating factors of site, lot, and day.

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February 24, 2011

Glossary of Symbols

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

| | | | | | |
|--|--|--|---|--|---|
| | Do Not Recycle | | Authorized Representative in the European Community | | Corrosive |
| | Use by or Expiration Date (Year-Month-Day) | | Contains Sufficient for "t" Tests | | Flammable |
| | Lot Number | | Upper Limit of Temperature | | Fragile, Handle with Care |
| | Serial Number | | Lower Limit of Temperature | | Keep Dry |
| | Catalog Number or Product Code | | Temperature Limitation | | This end up |
| | Attention: See Instructions for Use | | Consult Instructions for Use, "t" Version | | Get Green Point (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations. |
| | Manufacturer | | Infant | | |
| | In vitro Diagnostic Medical Device | | Harmful | | |

Revision History

| Date of Revision | Version | Description of Technical Changes* |
|------------------|---------|---|
| 2007-10-04 | 4.2 | <ul style="list-style-type: none"> Address Block: CHIRON Corporation changed to Novartis Vaccines and Diagnostics, Inc. |
| 2006-10-10 | 4.1 | <ul style="list-style-type: none"> Updated front page heading to contain full assay name from -aHCV to Anti-HCV Reagent Pack Corrected Revision History table -Date of Revision, Version 1.0 from 2005FEB08 to 2002FEB08 |
| 2005-11-22 | 4.0 | <ul style="list-style-type: none"> Changed "VITROS ECI" to "VITROS ECI/ECIQ". Intended Use: Broadened to include pregnant women. Removed text from Warning statement: "Assay performance characteristics have not been established for prenatal screening." Clinical Performance: added text and tables for Clinical Performance in Pregnant Women. Glossary of Symbols: updated table. Last page: added Chiron logo. Removed "Lots below Lot 5500 contain 250 mg." as no longer applicable |
| 2004-02-27 | 3.0 | <ul style="list-style-type: none"> Warnings and Precautions – Revised product handling precautions equivalent to Version 1.0 Copyright Table – Removed CE mark |
| 2003-11-07 | 2.0 | <ul style="list-style-type: none"> Warnings and Precautions – Updated product handling precautions. Add 2-Chloroacetamide to warning section. Glossary of Symbols – Added table and CE mark for IVDD. |
| 2002FEB08 | 1.0 | <ul style="list-style-type: none"> New format. Update to 2001AUG30 – Warnings and Precautions – Reagent Pack Contents |

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

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

Signature _____ Obsolete Date _____

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho-Clinical Diagnostics or its distributors. Copies of these are available on request.

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Novartis Vaccines and Diagnostics, Inc.
Emeryville, CA 94608-2018



Ortho-Clinical Diagnostics
Johnson & Johnson
50-100 Holmers Farm Way
High Wycombe
Buckinghamshire
HP12 4DP
United Kingdom



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February 24, 2011

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 (1).

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 (2).

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 Hepatitis Reference Laboratory (HRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a Visual Basic (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 lab supervisor. After each NHANES container is completed (i.e., when all clinical evaluations and analyses from each mobile survey site are complete), the supervisor will transmit the results to the SQL Server along with other NHANES IV data.

February 24, 2011

- C. Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly to tape 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 :L 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 4-8EC for no more than 5 days.
- I. For storage >5 days, samples are held at #-20EC. Samples held in long-term storage at #-20EC 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
 - (1) RIBA Processor System (Chiron Corporation, Emeryville, CA).

February 24, 2011

- (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

February 24, 2011

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 (15-30C°) 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).

February 24, 2011

- (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.

February 24, 2011

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 <u>single</u> 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 <u>two</u> 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.

February 24, 2011

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 more antigens present on the strip, the antigen bands may appear lighter than the background of the strip. Such bands should be interpreted as 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.

February 24, 2011

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:

| Intensity of Band | Score |
|--|-------|
| 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+ |

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

February 24, 2011

- 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

February 24, 2011

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 noninfected humans should have negative values for antibodies to hepatitis C.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25 EC 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 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). Generally, a CDC epidemiologist communicates the findings to other participants in the study. Final reports may be electronic or in printed form. All electronically held data are backed up routinely.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data.

Summary Statistics and QC graphs

Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

February 24, 2011

REFERENCES

1. 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.
2. 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.