VITROS Products
Immunodiagnostic

METHODOLOGY SHEET

aHCV
Anti-HCV

Vitros **Immunodiagnostic Products Anti-HCV Reagent Pack**

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 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.

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 prenatal screening or 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).

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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 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* ECi System. The amount of HRP conjugate bound is indicative of the level of anti-HCV present in the sample.

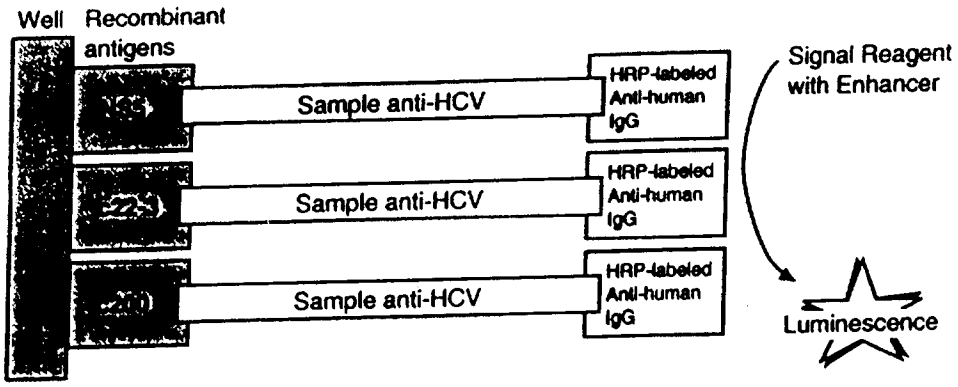
Assay Type

Immunometric assay

Assay Time and Temperature

Incubation time:	45 minutes
Time to first result:	55 minutes
Temperature:	37° C

Reaction Scheme



Warnings and Precautions

For in vitro diagnostic use only.

The *Vitros* Immunodiagnostic Products 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 inactivated (β-Propiolactone/Ultraviolet Irradiation).
- 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). Treat as if capable of transmitting infection.

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. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation.[5,6]

WARNING:

- ***The conjugate reagent in the Vitros Anti-HCV Reagent Pack contains ProClin 300 (1% w/w). The total ProClin 300 content is 260 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.[7]

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Reagents

Reagent Pack Contents

One *Vitros* Anti-HCV Reagent Pack, 100 tests (CAT No. 680 1325) contains:

- 100 coated wells [Hepatitis C virus recombinant antigens (NS5, c22-3, c200) derived from yeast (S.cerevisiae); coated at 0.41 µg/well]
- 24 mL assay reagent (buffer with 2- chloroacetamide anti-microbial agent)
- 26 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.

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* ECi 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 µL of sample for each determination.
- For details on minimum fill volume of sample cups or containers, refer to the *Vitros* ECi 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.

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Specimen Collection and Preparation (continued)

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 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

Materials Required But Not Provided

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

- *Vitros* ECi 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 System Operator's Guide for complete instructions on the operation of your *Vitros* ECi System.

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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 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 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 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 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 System Operator's Guide.
- 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* ECi 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.

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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* ECi System.

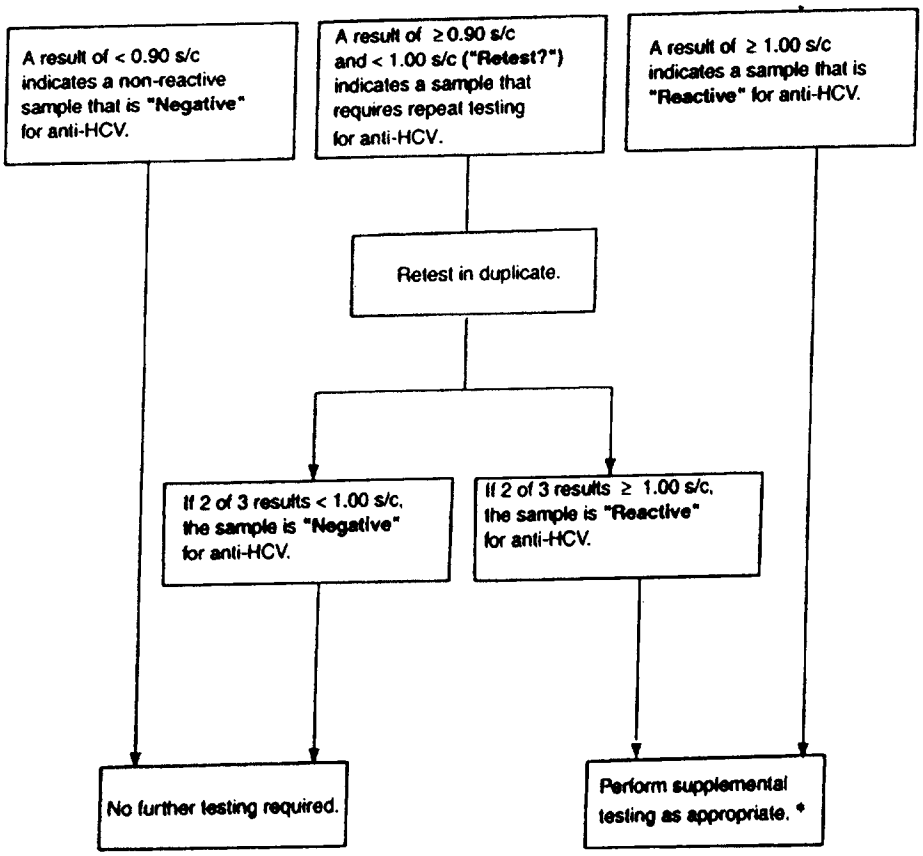
$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

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

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 1998;47 (RR-19)

Interpretation of Results and Expected Results (continued)

- 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 viral 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	93	75.6	123
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	6	100	6
90–100	F	0	0	0	0	0
	M	1	50.0	1	50.0	2
Total		412	23.9	1309	76.1	1721*

* Age was not reported for three subjects.

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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 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

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Performance Characteristics (continued)

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	87	0	0	6	1	94
IVDU, Current or Past	0	247	0	5	84	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 hemophilia 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.

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 AMPLICOR™ 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).

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Performance Characteristics (continued)

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
Hemophiliac*	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)	86.05–97.51
Dialysis Patients	98.39 (61/62)	91.34–99.96	97.24 (317/326)	94.82–98.73
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 (210/213)	95.94–99.71
Others/Unknown	100.0 (68/68)	94.72–100.0	99.22 (384/387)	97.75–99.84
None Specified	97.30 (36/37)	85.84–99.93	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.

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Performance Characteristics (continued)

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.

	Days to Evidence of HCV Infection									
	Reference Anti-HCV Assay		*Vitros* Anti-HCV Assay		HCV RNA*		Supplemental Testing†			Difference in Days to Anti-HCV Reactive Result
Panel ID	–‡	+**	–	+	–	+	–	IND ††	+	Reference – *Vitros*
6211	186	189	171	182	121	140	171	182	186	7
6212	37	53	0	12		0	14	23		41
6213	37	43	35	37	8	11	37		43	6
6214	32	49	25	30		0	25	30	49	19
6215	20		20			0	10	20		N/A
6216	17	23	17	23	17	23	17	23		0
6222	40		36	40	2	17	36	40		>0
6224	22		11	19		0	11	19		>3
6225	80		73	78	39	45	73	78		>2
6227	46	74	46	74	24	42	46		74	0
6228	38		24	28		0	28	31		>10
6229	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.

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Performance Characteristics (continued)

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	6	0	1	1	0	8
Dialysis Patients	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 Patients	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

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Performance Characteristics (continued)

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

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Performance Characteristics (continued)

Substances that do not Interfere

As recommended by NCCLS Protocol EP7[16], 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
Triolein	33.9 mmol/L	3000 mg/dL

Precision

Precision was evaluated on a different *Vitros* ECi 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.

	Mean *Vitros* Anti-HCV S/C (Ratio)	Within Day*		Between Day†		Total‡			
		SD	CV (%)	SD	CV (%)	SD	CV (%)	No. Observ.	No. Days
Site 1	0.14	0.004	2.8	0.012	8.7	0.013	9.1	40	20
	6.44	0.078	1.2	0.151	2.3	0.170	2.6	40	20
	1.06	0.027	2.5	0.085	8.0	0.089	8.4	40	20
Site 2	0.13	0.013	9.9	0.026	20.3	0.029	22.6	40	20
	6.35	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	8.6	40	20
	6.65	0.098	1.5	0.099	1.5	0.139	2.1	40	20
	1.07	0.017	1.6	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	18.6	162
0.90	0.000	0.0	0.000	0.0	0.055	6.1	162
1.02	0.017	1.7	0.000	0.0	0.076	7.5	162
4.78	0.088	1.8	0.270	5.6	0.333	7.0	162

* 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.

The data presented in both studies are a representation of assay performance based on the studies described. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect the reproducibility of assay results.

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Intended for Use in the United States

aHCV

Anti-HCV

VITROS Products
Immunodiagnostic

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

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Vitros Immunodiagnostic Products Anti-HCV Calibrator

JFC243/50.0

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

Intended Use

For use in the calibration of the *Vitros* ECi Immunodiagnostic System for the *in vitro* qualitative detection of immunoglobulin G antibody to hepatitis C virus (anti-HCV) in human serum and plasma (heparin, EDTA and sodium citrate) using *Vitros* Anti-HCV Reagent Packs. The *Vitros* Anti-HCV Calibrator has been validated for use only on the *Vitros* ECi Immunodiagnostic System with the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack. Refer to the *Vitros* Anti-HCV Reagent Pack instructions for use for further details.

Principles of Procedure

Calibration is lot specific; reagent packs and calibrators are linked by lot number. A Master Calibration is established for each new reagent lot by performing multiple assays. This is the process by which a lot-specific parameter [a] which links the cut-off value to the calibrator signal is determined.

Cut-off value = (a x Signal of CAL1).

The lot-specific parameter, the expected calibrator signal and the data which enables a System to calculate the cut-off value, are encoded on the lot calibration card. Scanning the lot calibration card loads the encoded data onto the System. When the calibrator is processed the validity of the calibration is assessed against a quality parameter which compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cut-off 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 control ranges to determine the validity of the calibration. Recalibration is required after a pre-determined calibration interval (refer to the *Vitros* Anti-HCV Reagent Pack calibration instructions), or when a different reagent lot is loaded.

Materials Provided

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

Reagent Preparation and Storage

The Anti-HCV Calibrator is supplied ready for use. Store unopened at 2-8 °C (36-46 °F). Do not use beyond the expiration date. After opening store for up to 13 weeks at 2-8 °C (36-46 °F) or 13 weeks at -20 °C (-4 °F) (with no more than 1 freeze-thaw cycle).

Quality Control and Procedural Notes

- 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 volume for a minimum of 6 calibration events.
- The Anti-HCV calibrator is automatically processed in duplicate.
- Evaporation will occur when calibrators are stored open on board the System, refer to the Operator's Guide, Chapter 6, Preparing Samples. Return to 2-8 °C (36-46 °F) as soon as possible after use, or load only sufficient volume for a single use. The calibrator may be aliquoted into alternative containers, which may be bar coded with the labels provided.

Procedure

For further information refer to the Anti-HCV Reagent Pack instructions for use. For detailed instructions on calibration refer to the *Vitros* Immunodiagnostic System Operator's Guide, Chapter 5, Performing Calibration.

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1. CDC-NIH. *Biosafety in Microbiological and Biomedical Laboratories – 3rd Edition.* HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C., 1993.
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Warnings and Precautions

For *In Vitro* Diagnostic Use Only

Warning - Potentially Infectious Material
The *Vitros* Anti-HCV 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 human immunodeficiency virus (HIV 1+2), using FDA approved methods (enzyme immunoassays). The HCV antibody positive plasma has been inactivated (β-Propiolactone/ Ultraviolet Irradiation).

HCV antibody negative plasma has been 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). Treat as if capable of transmitting infection.

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. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation.[1,2]

Warning - Contains Kathon
The *Vitros* Anti-HCV Calibrator contains Kathon (2%w/w). The total Kathon content is 40 mg. Kathon may cause sensitization by skin contact. Avoid contact with skin.

- R43 May cause sensitization by skin contact.*
- S24 Avoid contact with skin.*
- S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.*

UK: 'Chemicals (Hazard Information and Packaging for upply) Regulations 1994 (as amended)'.

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