

SUMMARY OF SAFETY AND EFFECTIVENESS

1. GENERAL INFORMATION

1.1. Name and Address of Applicant

Ortho-Clinical Diagnostics, Inc
100 Indigo Creek Drive
Rochester NY 14626-5101

1.2. Device Trade Name

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

1.3. Classification (Generic) Name of Device

Antibody to Hepatitis C Virus (Anti-HCV) Assay

1.4. PMA Number:

P010021

1.5. Date of Panel Recommendation:

Pursuant to Section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Microbiology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this Panel.

1.6. Date of Notice of Approval to Applicant: August 30, 2001

2. INDICATIONS FOR 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.

3. DEVICE DESCRIPTION

3.1. Principle of Device Methodology

The *Vitros* ECI Immunodiagnostic System (*Vitros* Analyzer) allows for the determination of analytes in human samples (serum and plasma). All assays on the *Vitros* Analyzer employ an enhanced chemiluminescence detection reaction. The Analyzer is fully automated with a refrigerated onboard assay storage system. All standard bar code symbologies are supported by the Analyzer, which has a throughput of up to 90 assays per hour. The Analyzer also provides menu driven software, which can be accessed, from a high-resolution touch screen monitor.

The *Vitros* Anti-HCV assay utilizes an immunometric technique, which involves a two-stage reaction. In the first stage, hepatitis C virus (HCV) antibody present in the sample binds with HCV recombinant antigens (c22-3, c200, and NS5) coated on the wells. Unbound sample is removed by washing. In the second stage, horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-human IgG) binds to any human IgG captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The *Vitros* Analyzer reads the light signals. The amount of HRP conjugate bound is indicative of the concentration of anti-HCV present.

3.2. Kit Configuration and Components

For detection of anti-HCV, the *Vitros* system is comprised of the following:

- *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack (*Vitros* Anti-HCV Reagent Pack) and *Vitros* Immunodiagnostic Products Anti-HCV Calibrator (*Vitros* Anti-HCV Calibrator) together comprise the *Vitros* Anti-HCV assay.

The *Vitros* Anti-HCV Reagent Pack is composed of three reagents:

- Conjugate reagent [HRP labeled mouse monoclonal anti-human IgG in buffered fetal calf serum with antimicrobial agent (ProClin 300)]
- Assay reagent [buffer with bovine serum albumin and antimicrobial agent (2-chloroacetamide)]
- Coated microwells [recombinant HCV antigens]

The *Vitros* Anti-HCV Calibrator contains:

- Inactivated anti-HCV positive human plasma in anti-HCV negative human plasma with antimicrobial agent (Kathon). The Calibrator is supplied ready for use.

In addition, the following components are required:

- *Vitros* ECi Immunodiagnostic System (*Vitros* Analyzer).
- *Vitros* Immunodiagnostic Products Signal Reagent and *Vitros* Immunodiagnostic Products Universal Wash Reagent.
- Quality control materials, e.g., *Vitros* Immunodiagnostic Products Anti-HCV Controls.

4. CONTRAINDICATIONS

For *in vitro* use only.

5. WARNINGS AND PRECAUTIONS

Warnings and precautions for users of the *Vitros* Anti-HCV Reagent Pack and Calibrator are stated in the respective product labeling.

6. ALTERNATE PRACTICES AND PROCEDURES

Determining the presence of anti-HCV in patients may be achieved by using a variety of commercially available, FDA licensed serological tests. Additionally, when these test results are used in combination with a physician's assessment and other laboratory test results, infection with HCV can be identified.

7. Marketing History

Countries where the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack and Calibrator have been or can be distributed through December 2000 are presented in the following table.

Country			
Argentina	Germany	Norway	Slovenia
Australia	Iceland	Panama	Spain
Belgium	India	Philippines	Sweden
Canada	Indonesia	Poland	Switzerland
Chile	Italy	Portugal	Taiwan
China	Japan	Romania	Thailand
Columbia	Korea	Russia	Turkey
Czech Republic	Malaysia	Saudi Arabia	UK
Denmark	Mexico	Singapore	
France	Netherlands	Slovak Republic	

This product has not been withdrawn from any of these markets for any reason.

8. Potential Adverse Effects of the Device on Health

Since the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack and *Vitros* Immunodiagnostic Products Anti-HCV Calibrator are for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient. However, failure of the product to perform as indicated or human error in use of the product may lead to a false result.

A false positive result using an anti-HCV assay is not considered a patient or public health concern since a reactive EIA result in a clinical lab should be followed up with supplemental tests (e.g., strip immunoblot assay (SIA) and/or PCR for detection of HCV RNA) to determine inactive or resolved infection versus active HCV replication.^{1,2} Treatment of the patient with chronic HCV infection is initiated only after extensive clinical, laboratory and behavioral assessment of the patient (e.g., elevated ALT levels for six months, detectable serum HCV RNA, liver biopsy with portal fibrosis, patient compliance, and abstinence from drugs and alcohol).³

A false negative anti-HCV result in a diagnostic setting may lead to a patient with HCV going unidentified. Under these circumstances, there is a safety concern for both the patient and the public, since such individuals may be capable of transmitting HCV infection. However, it has been recommended if a patient is known to be at high risk of HCV infection, or is symptomatic, and the physician's suspicion of HCV infection is high, HCV RNA testing is often employed and is of diagnostic value, even after an initial negative anti-HCV test result.²

9. Summary of Non Clinical Studies

9.1. Instrumentation

Software and hardware verification testing was performed for the *Vitros* ECI Immunodiagnostic System (*Vitros* Analyzer). Appropriate information and study results were furnished demonstrating that the *Vitros* Analyzer hardware and software, used with the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack, functioned as described and had appropriate safeguards

9.2. Comparison of Fresh Serum/Plasma Samples

To determine the acceptability of using the *Vitros* Anti-HCV assay for testing serum or plasma specimens, fifty fresh blood samples (25 unspiked and 25 spiked with anti-HCV to give a target result of 2.0 ± 1.0 signal/cut-off (s/c)), were collected and aliquoted into a variety of serum and plasma collection tubes. The following anticoagulants were evaluated in this study: sodium heparin, K₂ EDTA, sodium citrate. Testing with the *Vitros* Anti-HCV assay was conducted on the same day blood was drawn.

All unspiked samples, with serum and plasma (heparin, EDTA, and citrate) preparations, were classified correctly as anti-HCV negative in the *Vitros* Anti-HCV assay. All of the

¹ CDC, Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-Related Chronic Disease. MMWR 1998;47 (RR-19):1-39.

anti-HCV spiked samples tested maintained reactivity in the *Vitros* Anti-HCV assay regardless of the sample type preparation.

For matched anti-HCV spiked samples, EDTA plasma compared with serum showed -1.2% mean difference (n=25) in anti-HCV s/c ratio and heparin plasma compared with serum showed -0.4% mean difference (n=25) in anti-HCV s/c ratio. The mean s/c ratios for heparin and EDTA plasma were not statistically significant from the mean s/c ratio for serum. The mean s/c ratio (n=25) for citrate plasma was observed to be significantly lower than the mean s/c ratio for serum. In this study, the s/c ratio for a citrate plasma sample was approximately 20% lower than the corresponding serum samples. An appropriate warning has been placed into the *Vitros* Anti-HCV labeling to alert the user that citrate plasma specimens with high negative values (0.80 – 0.99 s/c) may require additional testing.

The study results with the appropriate labeling warning indicate that fresh serum and plasma (heparin, EDTA, and citrate) are suitable for use in the *Vitros* Anti-HCV assay.

9.3. Comparison of Stability of Serum/Plasma Samples

To determine whether plasma specimens had similar stability to serum specimens, twenty fresh blood samples (10 unspiked and 10 spiked with anti-HCV to give a target ratio of 2.0 ± 1.0 s/c), were collected and aliquoted into a variety of serum and plasma collection tubes. The following anticoagulants were evaluated in this study: sodium heparin, K₂ EDTA, and sodium citrate. Testing with the *Vitros* Anti-HCV assay was conducted on the same day blood was drawn (Day 0), and again after 5 and 7 days storage at 2-8 °C and after a minimum of 28 days at -20 °C.

None of the storage conditions tested had clinically significant effects on unspiked samples. All unspiked samples were classified correctly as negative, regardless of the storage condition. This is consistent with the combined regression results. There were no statistically significant positive slopes for any of the unspiked samples. The results are consistent with a negligible change in the s/c ratio from Day 0 to Day 28 (at -20 °C).

The stability of anti-HCV spiked samples stored at 2-8 °C was evaluated by determining the difference in s/c ratio between each sample tested at Day 0 and after storage at Day 5 and Day 7. For serum as well as all anticoagulants evaluated (heparin, EDTA and citrate), less than 10% change in s/c ratio (overall mean percentage difference) was observed between Day 0 and after 2-8 °C storage. The regression analyses suggest that EDTA and citrate plasma did not have statistically significant changes over 7 days and that although changes in serum and heparin were significant, the changes observed were within the precision of the assay and were not clinically significant.

For serum as well as all anticoagulants evaluated at -20 °C (heparin, EDTA, citrate), less than 10% change in s/c ratio (overall mean percentage difference) was observed between fresh samples (Day 0) and after storage for a minimum of 28 days. There was no statistical evidence of any change in the mean s/c ratios from Day 0 to Day 28 at -20 °C (serum, heparin, EDTA or citrate).

These data show that storage of serum or plasma (heparin, EDTA or citrate) samples for up to 5 days at 2-8°C, or 28 days at -20°C would not have a significant effect on the test results with the *Vitros* Anti-HCV assay.

9.4. Potentially Cross-Reacting Subgroups

To assess if there is a possibility of a false positive assay result due to other diseases or antibodies, the specificity of the *Vitros* Anti-HCV assay was evaluated by testing 292 samples from 22 potentially cross-reacting sub-groups. Patient samples from the following sub-groups were tested: HAV, HBV/HCV co-infection, HEV, non-viral liver disease, autoimmune disease (rheumatoid arthritis and systemic lupus erythematosus), CMV, EBV, HSV, parvovirus B19 virus infection, rubella, syphilis, toxoplasmosis, HIV 1/2 antibody positive, HTLV 1/2 antibody positive, recent influenza vaccine recipients, heterophilic antibodies (human anti-mouse), yeast infection, multiparous females, multiple transfusion patients, dialysis patients and HBV/HDV co-infection. Ten (10) individuals reported to be co-infected with HBV and HCV and two (2) individuals reported to be co-infected with HBV and HDV were observed to be reactive in both the *Vitros* Anti-HCV assay and in an FDA licensed reference assay. For the two specimens reported to be co-infected with HBV and HDV, no further testing was performed to confirm the anti-HCV positive results.

Of the 22 sub-groups evaluated, 280 out of 280 samples that tested negative in an FDA licensed assay were observed to be negative in the *Vitros* Anti-HCV assay.

9.5. Genotype Detection

The ability of the *Vitros* Anti-HCV assay to detect antibodies to various HCV genotypes was assessed using the Boston Biomedica, Inc. Worldwide HCV Performance Panel. The panel consisted of 20 human plasma samples that were pre-determined by the supplier to include four of the six recognized genotypes of HCV (1, 2, 3, and 4). 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 in the *Vitros* Anti-HCV assay. In additional studies, 7/7 samples characterized to be genotype 5 by the supplier tested *Vitros* Anti-HCV reactive, while 1/1 sample characterized as genotype 6 was *Vitros* Anti-HCV reactive.

9.6. Interfering Substances

To determine the effect of hemolysis, high bilirubin levels, and specimens containing high levels of glycerides, spiked specimens containing purified hemoglobin, bilirubin, and triolein were evaluated using samples from 10 blood donors. The results (test results at each level of interferent) demonstrate that hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL), and triolein (up to 3000 mg/dL) cause no misclassification of results.

Samples spiked with anti-HCV to give a target result of 1.5 - 2.0 s/c were observed to remain reactive at all levels tested with each potential interferent. Similarly, no interference was observed in samples not spiked with anti-HCV (Negative).

9.7. Stability

Vitros Anti-HCV Reagent Packs, Calibrators and Controls that were subjected to a period of simulated transport to mimic effects of shipment were tested at various time points up to 52 weeks after storage at 2-8°C. In addition, three commercially obtained performance panels were tested using transported, stored materials at weeks 0, 26, and 52. Materials stored for 52 weeks yielded results that indicated that there was no trend in QC In-house Control results or *Vitros* Anti-HCV Control results observed over the storage period, and there was no change in the classification of results obtained for the performance panel samples at week 26 and 52 relative to week 0 for all Kit Lots tested.

This data supports the storage of the *Vitros* Anti-HCV Reagent Pack, Calibrator and the *Vitros* Anti-HCV Controls for 52 weeks at 2-8°C. However, preservative effectiveness testing on the Calibrator indicates acceptable results up to week 35 only. Therefore, based on the data currently available the shelf-life of the *Vitros* Anti-HCV Calibrator will be set at 35 weeks, while the shelf-life of the *Vitros* Anti-HCV Reagent Pack will be set at 52 weeks.

9.8. Open On-Board Storage for the *Vitros* Anti-HCV Reagent Pack

Vitros Anti-HCV Reagent Packs that were subjected to a period of simulated transport to mimic effects of shipment were opened and placed in an environmental chamber for a period of 8 weeks to simulate the storage on board the *Vitros* Analyzer. These Reagent Packs were tested at various time points within the 8-week period. In addition, a single transported, opened Reagent Pack from each Kit Lot was removed from the chamber on 6 different occasions, and brought to room temperature over the 8 week period to simulate typical customer usage.

Results of testing were within acceptability limits, overall no trend was observed between freshly opened Reagent Packs stored at 2-8°C and Reagent Packs stored opened on-board for 8 weeks. This data supports the on-board storage of Reagent Packs for up to 8 weeks.

9.9. Open Off-Board Storage for the *Vitros* Anti-HCV Calibrators

To determine the effects of various normal use storage conditions the *Vitros* Anti-HCV Calibrators that were subjected to a period of simulated transport to mimic effects of shipment were opened, pooled, sub-aliquoted and stored at 2-8°C and -20°C for 13 weeks. Results of testing these Calibrators at various time points up to 13 weeks indicated no observable trends and met all acceptance criteria.

The data supports the storage of the Calibrator at 2-8°C and -20°C after opening for up to 13 weeks.

9.10. Microbiology

A study was done to assess the ability of the anti-microbial agents (2-chloro-acetamide, ProClin 300 and Kathon) contained in the *Vitros* Anti-HCV reagents to provide protection against adventitious contamination by microorganisms. Evaluation of the microbial load of each reagent (Assay Reagent, Conjugate Reagent, and Calibrator) post-dispensing and at 52 weeks demonstrated that the total aerobic count is generally on the order of <10CFU/mL. In addition, the levels of preservative in each reagent were determined over a period of at least 52 weeks. For the Assay Reagent, the preservative concentration remained constant throughout 56 weeks of testing. Results for the Conjugate Reagent and Calibrator demonstrated that the preservative concentrations were above the minimum inhibitory concentration throughout 58 and 45 weeks of testing, respectively.

A study conducted according to US Pharmacopoeia (USP) 23/NF 18, general chapter 51, assessed the ability of the reagents to withstand or control microbial contamination. Results indicated that the preservative systems for each reagent met the requirement of the USP 23 at 53 weeks for Assay Reagent, 52 weeks for Conjugate Reagent, and 35 weeks for the Calibrator, based on the date of manufacture for each reagent.

9.11. Precision - Reproducibility

To determine the extent to which replicate analyses agreed with each other precision was evaluated on a different *Vitros* ECI Immunodiagnostic System at three external sites, using one reagent pack and calibrator kit lot. At least 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.

Clinical Site	Mean <i>Vitros</i> aHCV S/C (Ratio)	Within day *		Between day †		Total ‡		No. Obs.	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
	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.

Reproducibility was 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 <i>Vitros</i> Anti-HCV S/C (Ratio)	Between Site *		Between Lot †		Total ‡		No. Obs.
	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: Variability of the assay incorporating factors of site, lot and day.

9.12. Calibration Interval

To determine the *Vitros* ECi System's calibration interval for the *Vitros* Anti-HCV assay the performance of the *Vitros* Anti-HCV assay within one calibration interval (28-days) was evaluated at three sites by testing a three member panel with one kit lot. One panel member was close to the *Vitros* Anti-HCV assay cutoff. Additional testing was performed on Day 29 (study day 30) of the calibration cycle to show that the Analyzer would still yield valid results beyond the end of a 28-day cycle. Two replicates of each panel member were run per day at each clinical site. Appropriate calibration was performed and verified on Day 1 of the study, and the testing was performed for a total of 20 study days over a 30-day period.

Least squares regression analyses were performed within site and across sites. For analyses within site, although each panel member showed a statistically significant slope at one of the three sites, the changes in S/C ratios over the entire testing period were so small that they would not have any clinical implications. For analyses across sites, the slope was statistically significant only for the negative panel member. The negative slope observed would not be clinically relevant over time, however.

The *Vitros* Anti-HCV assay demonstrated adequate performance throughout the entire calibration interval (28-days), and continued to perform successfully one day beyond the expiration of calibration.

9.13. Seroconversion Panels

To assess the ability of the *Vitros* Anti-HCV assay to detect HCV seroconverters 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.

Panel ID	Days to Evidence of HCV Infection									Difference in Days to Anti-HCV Reactive Result Reference <i>Vitros</i>
	Reference Anti-HCV Assay		<i>Vitros</i> Anti-HCV Assay		HCV RNA ¹		Supplemental Testing ²			
	- ³	+ ⁴	-	+	-	+	-	IND ⁵	+	
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	4	7		0	4	7		0
SC-0100	0	7		0				0		7

¹ Research assay not verified for clinical use.

² Chiron**RIBA**HCV 3.0 SIA.

³ Post bleed day of last nonreactive result, usually denotes previous bleed from first reactive.

⁴ Post bleed day of first reactive result.

⁵ Post bleed day of first indeterminate result.

The *Vitros* Anti-HCV assay was reactive in the same bleed as the reference assay in 5 (25%) of the 20 panels tested. The *Vitros* assay was reactive by as many as 6 bleeds and 41 days earlier than the reference assay in 14 (70%) panels. The reference anti-HCV assay remained negative in four of these 14 panels. Neither assay was reactive throughout one panel.

10. Summary of Clinical Studies

To evaluate the *Vitros* Anti-HCV assay's ability to detect anti-HCV antibody in a group of individuals that would normally be tested in a clinical situation a multi-center prospective study was conducted to evaluate the clinical performance of the *Vitros* Anti-HCV assay. The study population included individuals with specific risks or history associated with HCV infection including transfusions or transplants before 1992, past and current use of intravenous drugs, 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% at two sites). 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 was 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 the Chiron*RIHA*HCV 3.0 SIA, when required. In addition, reference assays for HBsAg, HBsAg Confirmatory, and IgM anti-HAV 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%).

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 2 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 repeatedly 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 distribution of *Vitros* Anti-HCV repeatedly reactive and negative results among the study subjects without signs or symptoms of hepatitis by age and gender are presented in the following table.

Expected Results for the *Vitros* Anti-HCV Assay in Study Subjects Without Signs or Symptoms of Hepatitis

Expected Results for the Vitros Anti-HCV Assay in Study Subjects Without Known HCV Infection						
Age Range	Gender	Vitros Anti-HCV Result				Total
		Reactive		Negative		
		N	Percent	N	Percent	
0-9	Female	0	0	0	0	0
	Male	11	78.6	3	21.4	14
10-19	Female	0	0.0	21	100	21
	Male	22	68.8	10	31.3	32
20-29	Female	7	5.4	122	94.6	129
	Male	30	24.4	93	75.6	123
30-39	Female	20	12.7	137	87.3	157
	Male	78	30.0	182	70.0	260
40-49	Female	37	21.4	136	78.6	173
	Male	111	39.8	168	60.2	279
50-59	Female	20	15.0	113	85.0	133
	Male	41	29.5	98	70.5	139
60-69	Female	11	12.4	78	87.6	89
	Male	14	20.0	56	80.0	70
70-79	Female	3	6.7	42	93.3	45
	Male	6	15.4	33	84.6	39
80-89	Female	0	0.0	10	100	10
	Male	0	0.0	6	100	6
90-100	Female	0	0	0	0	0
	Male	1	50.0	1	50.0	2
Total		412	23.9	1309	76.1	1721 ¹

¹ Age was not reported for three subjects.

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 following table.

Assignment of HCV Status to the Study Population

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	HCV Status Cannot be Determined (Not Determined)

The following table compares *Vitros* Anti-HCV 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). Assignment of HCV status was according to the algorithm presented in the previous table.

Comparison of *Vitros* Anti-HCV Results and HCV Status Among Study Subjects Ranked According to the Risk for HCV Infection (N=2622)

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 Patient	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
Other/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 the 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 presented below.

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.

<i>Vitros</i> 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 Patient
			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 patient
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)
† 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)
** Indicates active HCV infection

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 the 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 Patient	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 assay 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.

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)

(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	Repeatedly Reactive	Negative	Repeatedly Reactive	Negative	Repeatedly Reactive	
IVDU, current or past	0	6	0	1	1	0	8
Dialysis Patient	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)

(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	Repeatedly Reactive	Negative	Repeatedly Reactive	Negative	Repeatedly Reactive	
IVDU, current or past	0	0	0	0	0	0	0
Dialysis Patient	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

11. Conclusions Drawn from Studies

It is believed the previous studies demonstrate the following:

- Acceptable performance is obtained with the *Vitros* Anti-HCV assay when testing specimens collected in serum or plasma (heparin, EDTA or sodium citrate).
- The *Vitros* Anti-HCV assay recognizes all genotypes represented in the Boston Biomedica, Inc. Worldwide HCV Performance Panel (1 through 4) as well as genotypes 5 and 6 tested separately.
- The *Vitros* Anti-HCV Reagent Pack can be stored for up to 52 weeks at 2-8°C while the *Vitros* Anti-HCV Calibrator can be stored for up to 35 weeks at 2-8°C. After opening,

the Reagent Pack can be stored on-board the *Vitros* Analyzer (4-8°C, ≤40% relative humidity) for up to 8 weeks, and the Calibrator stored for up to 13 weeks at 2-8°C or -20°C (with no more than one freeze-thaw cycle).

- The preservative systems that the *Vitros* Anti-HCV assay reagents contain have been shown to meet USP 23 requirements at 53 weeks for the Assay Reagent, 52 weeks for the Conjugate Reagent, and 35 weeks for the Calibrator.
- The *Vitros* Anti-HCV assay demonstrated precision estimates of <20% within day and between day for each site as well as across all sites, and <5% between replicate, <3% between day, <5% between site, and <20% between lot when these variables were introduced.
- The *Vitros* Anti-HCV assay has been shown to perform adequately over a 28-day period on a single stored calibration.
- Based on the results of the clinical laboratory studies, the *Vitros* Anti-HCV assay, when used according to the provided directions, and in conjunction with other laboratory results and clinical information, is safe and effective in providing 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.

Safety

As a diagnostic test, the *Vitros* Anti-HCV assay involves removal of blood from an individual for testing purposes. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood is removed.

Benefit/Risk

The submitted clinical studies have shown that the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack, when compared to reference clinical laboratory procedures, has a similar ability to detect the presence of immunoglobulin G anti-HCV in specimens from individuals infected with HCV (state or associated disease not determined). The rate of false positivity and false negativity are within acceptable limits compared to the reference assay. It has been shown that the device has no demonstrable cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, this device should benefit the physician in the diagnosis of HCV associated and nonassociated hepatitis.

Based on the results of the preclinical and clinical laboratory studies the *Vitros* Immunodiagnostic Products Anti-HCV Reagent Pack, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and effective and pose minimal risk to the patient due to false test results.

12. CDRH Decision

FDA issued an approval order on August 30, 2001.

The applicant's manufacturing facility inspected on February 15, 2000 and was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

13. Approval Specifications

Directions for use: See Labeling

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.