SUMMARY OF BASIS

FOR APPROVAL

Proper Name: Hepatitis C Virus Encoded Antigen (Recombinant c33c and NS5 antigens; Synthetic 5-1-1, c100, and c22 peptides)

Trade Name: CHIRON® RIBA™ HCV 3.0 Strip Immunoblot Assay

Applicant: Chiron Corporation
4560 Horton Street
Emeryville, CA 94608

Reference Numbers: 96-0403
96-0758

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I. INDICATIONS FOR USE

The CHIRON® RIBA™ HCV 3.0 SIA is an *in vitro* qualitative enzyme immunoblot assay for the detection of antibodies to individual proteins encoded by the hepatitis C virus (anti-HCV) in human serum or plasma. It is intended for use as an additional, more specific test on human serum or plasma specimens found to be repeatedly reactive using a licensed anti-HCV screening procedure, such as Enzyme-Linked Immunosorbant Assay (ELISA). Additionally, the CHIRON® RIBA™ HCV 3.0 SIA may be used as an aid in the differential diagnosis of patients with biochemical evidence of hepatitis.

II. BRIEF DESCRIPTION OF THE TEST KIT

The CHIRON® RIBA™ HCV 3.0 SIA is an *in vitro* qualitative immunoblot assay which utilizes recombinant HCV-encoded antigens and synthetic HCV-encoded peptides immobilized as individual bands onto test strips. The two recombinant antigens (c33c and NS5) and two of the synthetic peptides (c100p and 5-1-1p) are derived from putative nonstructural regions of the virus, while the third peptide (c22p) corresponds to the putative nucleocapsid (core) viral protein. Since the recombinant HCV c33c and NS5 antigens are produced as individual fusion proteins with human superoxide dismutase (hSOD), recombinant hSOD has also been included as a control band on the strip. The hSOD control band enables detection of antibodies against hSOD which are not specific for the HCV-encoded portions of the recombinant HCV antigens. The HCV c33c Antigen is produced in genetically engineered bacteria (*E. coli),* while the HCV NS5 Antigen and hSOD are produced in genetically engineered yeast (*S. cerevisiae).*

The CHIRON® RIBA™ HCV 3.0 Strip Immunoblot Assay contains the following components:

Component Description	Quantity Provided
Hepatitis C Virus (HCV) Encoded Antigen (Recombinant c33c and NS5 antigens; Synthetic 5-1-1, c100, and c22 peptides) 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: Provided in 6 sealed pouches; each pouch contains 5 strips in separate tubes.
Specimen Diluent: phosphate-buffered saline (PBS) with bovine protein stabilizers and detergents. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	1 bottle containing 100 mL
Conjugate: peroxidase-labeled goat antihuman IgG (heavy and light chains), with bovine protein stabilizers. Contains 0.01% thimerosal as a preservative.	1 bottle containing 65 mL
Substrate Solution: 4-chloro-1-naphthol in methanol.	1 bottle containing 12 mL
Substrate Buffer: phosphate-buffered hydrogen peroxide.	1 bottle containing 60 mL
Wash Buffer Concentrate (50x): phosphate-buffered detergent solution containing 0.01% thimerosal as a preservative.	1 bottle containing 80 mL
Positive Control (Human): inactivated human serum or plasma containing antibodies to HCV (anti-HCV); nonreactive for hepatitis B surface antigen (HBsAg), antibodies to human immunodeficiency virus type 1 (anti-HIV-1) and type 2 (anti-HIV-2), and antibodies to human T lymphotropic virus type I (anti-HTLV-I) and type II (anti-HTLV-II) when tested by FDA-licensed assays. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	One vial containing 0.3 mL
Negative Control (Human): human serum or plasma nonreactive for HBsAg, anti-HIV-1, anti-HIV-2, anti-HCV, anti-HTLV-I, and anti-HTLV-II when tested by FDA-licensed assays. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	One vial containing 0.3 mL

III. MANUFACTURING AND CONTROLS

A. Description of the Manufacturing Process

The CHIRON® RIBA™ HCV 3.0 Strip Immunoblot Assay (SIA) is prepared under U.S. License Number 1106 in Chiron Corporation facilities located in Emeryville, California.

Two recombinant HCV-encoded antigens and three synthetic HCV-encoded peptides are the basis for the coated test strips in the CHIRON® RIBA™ HCV 3.0 SIA. The two recombinant antigens (c33c and NS5) and two of the synthetic peptides (c100p and 5-1-1p) are derived from putative nonstructural regions of the virus, while the third peptide (c22p) corresponds to the putative nucleocapsid (core) viral protein. Since the recombinant HCV c33c and NS5 antigens are produced as individual fusion proteins with human superoxide dismutase (hSOD), recombinant hSOD has also been included as a control band on the strip. The HCV c33c Antigen is produced in genetically engineered bacteria (*E. coli*), while the HCV NS5 Antigen and hSOD are produced in genetically engineered yeast (*S. cerevisiae*).

Positive and Negative Controls are prepared from human serum or plasma which are positive and negative, respectively, for anti-HCV. The positive serum or plasma is inactivated by a combination of physical (heat) and chemical/ultraviolet (UV) treatment.[1,2]

Raw materials intended for use in the product are subjected to appropriate quality control evaluations before they are accepted for use in manufacturing. Acceptance criteria and performance specifications have been established for all test kit components. Components are assembled into test kits, each lot of which subjected to a final performance test. Each lot of CHIRON® RIBA™ HCV 3.0 SIA is tested with an in-house panel

of specimens with varying levels of anti-HCV reactivity and must meet the performance requirements of the panel. The CHIRON® RIBA™ HCV 3.0 SIA meets the FDA potency requirements.

B. Stability Studies

The stability of the CHIRON® RIBA™ HCV 3.0 SIA has been established based upon testing at the recommended storage conditions of 2 to 8°C. The stability test results indicate no compromise in product performance and support a 14-month dating period for the test kit. The expiration date of the lot is the same as that of the shortest dated kit component.

C. Methods of Validation

Production of test kit components is monitored by in-process testing. Product purity and potency are assured through evaluation of product appearance, bioburden tests, and performance. Product performance is assessed through laboratory evaluations of each test kit against an in-house panel containing specimens that are reactive for anti-HCV and specimens that are non-reactive for anti-HCV. The CHIRON® RIBA™ HCV 3.0 SIA meets the FDA potency requirements.

Each lot of product and protocols summarizing pertinent product testing are submitted for evaluation and approval by FDA prior to release for distribution.

D. Labeling

The product labeling, including immediate container and package labels and the package insert (directions for use), have been reviewed for compliance with 21 CFR 610.60, 610.61, 610.62, and 809.10 and found to be satisfactory. The package insert states that the CHIRON® RIBA™ HCV 3.0 SIA is a qualitative test for the detection of anti-HCV in human

serum or plasma. The product tradename, CHIRON® RIBA™ HCV 3.0 Strip Immunoblot Assay, is not known to conflict with any other biologic or device tradename.

E. Establishment Inspection

A Pre-license Inspection of the areas where hepatitis products are manufactured, tested, stored, and shipped was conducted on January 25, 26, 27, 28 and 29, 1999. Facilities and procedures were found to comply with current good manufacturing practices.

F. Environmental Impact Analysis Report (EIAR)

A detailed EIAR was filed by the manufacturer. This product has no significant environmental impact. A summary of the procedures taken by the manufacturer to protect the environment are stated as follows:

1. Positive control human serum/plasma is heat chemically/ultraviolet (UV)-treated (inactivated) before being used to manufacture the Positive Control.[1,2]
2. All biohazardous waste material is disposed of as if it contains infectious agents.

All applicable federal, state, and local environmental regulations are being met by the manufacturer.

References

1 Redfield, D.C., Richman, D.D., Oxman, M.N., Kronenberg, L.H. Psoralen inactivation of influenza and herpes simplex viruses and of virus-infected cells. Infection and Immunity 32, 1216-1226 (1981).

2 Swanstrom R., Hallick, L.M., Jackson, J., Hearst, J. E., Bishop, J.M. Interaction of psoralen derivatives with the RNA genome of Rous sarcoma virus. Virology 113, 613-622 (1981).

IV. BIOLOGICAL PRINCIPLES OF THE TEST

A. Chemical and Biological Principles of the Procedure

The CHIRON® RIBA™ HCV 3.0 SIA is a three-stage test which utilizes individual recombinant HCV antigens and synthetic peptides immobilized as individual bands onto the test strips.

In the first stage, the specimen or assay control is diluted and incubated with the strip. Antibodies specific to HCV, if present, will bind to the corresponding recombinant antigen and/or synthetic peptide bands on the strip. Removal of unbound serum/plasma components is accomplished by aspiration and washing.

In the second stage, the strip is incubated in the presence of a peroxidase-labeled goat anti-human IgG conjugate. The conjugate binds to the human IgG portion of the antigen-antibody complexes. Removal of unbound conjugate is accomplished by decantation and subsequent wash steps.

In the third stage, a colorimetric enzyme detection system composed of hydrogen peroxide and 4-chloro-1-naphthol is added. If bound conjugate is present, the enzymatic reaction will produce an insoluble blue-black colored reaction product at each specific HCV antigen, peptide or control band. The color reaction involves the initial divalent oxidation of the peroxidase enzyme by hydrogen peroxide. Subsequent reduction of peroxidase to its initial state by two successive univalent interactions with soluble 4-chloro-1-naphthol results in the insoluble blue-black colored reaction product. After the development of color on the strip, the reaction is stopped by removal of the reactants and final wash steps. The visual band patterns which develop on each individual strip are the result of specific antibody being bound to each of the individual recombinant antigens and/or synthetic peptides on that strip. The reactivity of specimens towards each antigen band is determined by visually comparing

the intensity of the individual antigen band with that of the low and high human IgG internal control bands blotted onto each strip.

B. Preclinical Data

Prior to initiation of clinical studies, the CHIRON® RIBA™ HCV 3.0 SIA was evaluated at Chiron Corporation with serum specimens from the following groups: RIBA™ HCV 2.0 SIA four band reactive specimens, dilutions of RIBA™ HCV 2.0 SIA four band reactive specimens, plasma donor HCV seroconversion panels, non transfusion associated HCV seroconversion cases, high risk (hemophiliacs, intravenous drug abusers and renal dialysis patients), RIBA™ HCV 2.0 SIA indeterminate specimens from high risk donors (hemophiliacs and renal dialysis patients), RIBA™ HCV 2.0 SIA indeterminate specimens from the Chiron Reference Laboratory and low risk (presumably healthy volunteer blood donors). The results of these in-house studies are described below.

1. RIBA™ HCV 2.0 SIA Four Band Reactive Specimens

A total of 108 specimens which exhibited reactivity toward all 4 HCV antigens in the RIBA™ HCV 2.0 SIA were tested. Given the reactivity pattern, these specimens are expected to represent true HCV infections. A summary of the RIBA™ HCV 3.0 SIA results for these 108 specimens is presented in Table 1. A total of 108/108 (100%) of the specimens contained antibody toward the c100/5-1-1 peptides, recombinant c33c and c22 peptide, while 83/108 (76.9%) of the specimens contained antibody toward recombinant NS5. All specimens included in this study with antibody reactivity toward the recombinant antigens, 5-1-1, c100-3, c33c, and c22-3 in the RIBA™ HCV 2.0 SIA also exhibited reactivity toward the c100/5-1-1 and c22 peptides and recombinant c33c in the RIBA™ HCV 3.0 SIA. These results demonstrate that the RIBA™ HCV 3.0 SIA detects

antibody responses to the HCV antigens present in the RIBA™ HCV 2.0 SIA (5-1-1, c100-3, c33c and c22-3) in addition to NS5.

Table 1

Summary of RIBA™ HCV 3.0 SIA Results for
RIBA™ HCV 2.0 SIA 4 Band Reactive Specimens

Antigen Reactivity	# / # Tested (% Frequency)
c100p/5-1-1p	108/108 (100.0%)
c33c	108/108 (100.0%)
c22p	108/108 (100.0%)
NS5	83/108 (76.9%)
Total Number Tested	108 (100.0%)

2. Dilutions of RIBA™ 2.0 HCV SIA Four Band Reactive Specimens

In this small population, the RIBA™ 3.0 HCV SIA showed higher dilutional sensitivity than the RIBA™ HCV 2.0 SIA in 7/10 cases (70%). The two assays showed equal dilutional sensitivity in 2/10 cases (20%) and the RIBA™ HCV 2.0 SIA showed higher dilutional sensitivity in 1/10 cases (10%). In the seven cases where the RIBA™ HCV 3.0 SIA possessed higher dilutional sensitivity, one additional serial dilution was detected in five cases and two additional serial dilutions were detected in two cases. In the single case where the RIBA™ HCV 2.0 SIA demonstrated higher dilutional sensitivity, one additional serial dilution was detected. The results are shown in Table 2.

Table 2

RIBA™ HCV 2.0 SIA Four Band Reactive Samples (N=10)
Dilutional Sensitivity of RIBA™ HCV 2.0 and RIBA™ HCV 3.0 Strip Immunoblot Assays

	LAST DILUTION WITH AT LEAST ONE REACTIVE ANTIGEN		ANTIGENS DETECTED IN LAST DILUTION	
SAMPLE	RIBA™ 2.0 SIA	RIBA™ 3.0 SIA	RIBA™ 2.0 SIA	RIBA™ 3.0 SIA
745	1:640	1:320	c22-3	c33c, c22p, NS5
751	1:640	1:1280	c33c	NS5
757	1:320	1:1280	c33c	NS5
765	1:160	1:320	c22-3	c100p/5-1-1p, c33c
287	1:320	1:1280	c33c, c22-3	NS5
292	1:320	1:640	c33c	c33c
305	1:40	1:80	c22-3	c100p/5-1-1p, c33c
306	1:80	1:80	5-1-1, c100-3	c100p/5-1-1p, c33c
308	1:80	1:80	c22-3	c100p/5-1-1p, c33c
681	1:80	1:160	c33c, c22-3	c33c, c22p

3. Plasma Donor HCV Seroconversion Panels

Four panels of specimens containing serial bleeds from plasma donors were tested in the RIBA™ HCV 3.0 SIA. In three panels, A, B, and C, seroconversion to reactivity for one antigen occurred earlier in the RIBA™ HCV 3.0 SIA than in the RIBA™ HCV 2.0 SIA. In a single panel (Panel D) seroconversion was observed to occur simultaneously in both assays. Seroconversion was observed in the RIBA™ HCV 3.0 SIA 7 days earlier in Panel C, 20 days earlier in Panel A, and at least 71 days earlier in Panel B. In two of the three panels, in which seroconversion was detected earlier by the RIBA™ HCV 3.0 SIA (Panels A and C), the first reactive antigen was c33c. In three of four panels, the first reactive antigen in the RIBA™ HCV 3.0 SIA was c33c.

4. Non-Transfusion Associated HCV Seroconversion Cases

Eighteen panels of specimens containing serial bleeds from non transfusion transmitted Non A, Non B Hepatitis cases were tested in the RIBA™ HCV 3.0 SIA. A summary of the data appears in Table 3. In sixteen of the eighteen panels, the RIBA™ HCV 3.0 SIA became reactive earlier than the RIBA™ HCV 2.0 SIA for either a single antigen or two or more antigens. In two cases both the RIBA™ HCV 3.0 and RIBA™ HCV 2.0 SIA became reactive for two or more antigens at the same time and neither assay was reactive to a single antigen in any of the available specimens. In no cases did the RIBA™ HCV 2.0 SIA become reactive for a single antigen or two antigens before the RIBA™ HCV 3.0 SIA. These data demonstrate the increased sensitivity of the RIBA™ HCV 3.0 SIA over the RIBA™ HCV 2.0 SIA.

In 17/18 (94.4%) of the seroconversion panels, c33c was either the first or one of the first two reactive antigens in the RIBA™ HCV 3.0 SIA. All of the other antigens used in the RIBA™ HCV 3.0 SIA, however, were also useful in the early detection of seroconversions. For example, the mixture of c100/5-1-1 peptide, was the first reactive antigen or one of the first two reactive antigens in 9/18 (50.0%) of the cases. This was also the case for the c22 peptide (c22p). Recombinant NS5 was the first reactive antigen, or one of the first two reactive antigens in 2/18 (11.1%) of the cases.

Table 3

NT-NANB Seroconversion Panels Summary

Seroconversion Series	RIBA 2.0 SIA		RIBA 3.0 SIA	
	First Bleed Reactive for One Antigen	First Bleed Reactive for Two or More Antigens	First Bleed Reactive for One Antigen	First Bleed Reactive for Two or More Antigens
DV031185	10/02/80 c22-3 2+	No Bleed Reactive for Two Antigens	No Bleed Reactive for a Single Antigen	10/02/80 c33c 1+ c22p 3+
DV091383	11/17/81 c33c 1+	No Bleed Reactive for Two Antigens	10/20/81 c33c 3+	12/15/81 c100p/5-1-1p 2+ c33c 4+
CSH15266	No Bleed Reactive for a Single Antigen	1/08/81 c33c 1+ c22-3 4+	No Bleed Reactive for a Single Antigen	1/08/81 c100p/5-1-1p 1+ c33c 4+ c22p 4+ NS5 1+
DV361037	10/02/80 c22-3 2+	No Bleed Reactive for Two Antigens	9/06/80 c22p 2+	No Bleed Reactive for Two Antigens
DV091392	4/24/81 c33c 1+	6/08/81 5-1-1 2+ c33c 3+	4/06/80 c33c 4+	6/08/81 c100P/5-1-1p 3+ c33c 4+
DV011256	6/10/81 5-1-1 2+	8/13/81 5-1-1 4+ c33c 2+	No Bleed Reactive for a Single Antigen	6/10/81 c100p/5-1-1p 2+ c33c 3+
DV141019	No Bleed Reactive for a Single Antigen	8/21/81 c33c 1+ c22 1+	No Bleed Reactive for a Single Antigen	8/12/81 c33c 3+ c22p 1+
DV161123	7/06/81 c22-3 4+	8/31/82 c100-3 1+ c33c 2+ c22-3 4+	8/12/80 No Bleed Reactive for a Single Antigen	7/06/81 c33c 2+ c22p 4+
DV101080	9/16/81 c100-3 1+	No Bleed Reactive for Two or More Antigens	No Bleed Reactive for a Single Antigen	9/16/81 c33c 4+ c22p 2+

Table 3 (cont.)

	RIBA 2.0 SIA		RIBA 3.0 SIA	
Seroconversion Series	First Bleed Reactive for One Antigen	First Bleed Reactive for Two or More Antigens	First Bleed Reactive for One Antigen	First Bleed Reactive for Two or More Antigens
DV41005	4/03/82 5-1-1 2+	3/22/85 5-1-1 1+ c33c 2+ c22-3 4+	No Bleed Reactive for a Single Antigen	4/03/82 c100p/5-1-1p 2+ c33c 2+
DV061378	11/24/80 c33c 1+	2/11/81 5-1-1 2+ c33c 4+	No Bleed Reactive for a Single Antigen	11/24/80 c100p/5-1-1p 2+ c33c 4+
DV061395	No Bleed Reactive for a Single Antigen	12/16/80 5-1-1 1+ c100-3 1+	12/04/80 c33c 1+	12/16/80 c100p/5-1-1p 2+ c33c 3+
DV081265	5/30/81 c33c 1+	8/10/81 5-1-1 2+ c100-3 2+ c33c 4+	No Bleed Reactive for a Single Antigen	5/30/81 c100p/5-1-1p 3+ c33c 4+
DV361017	4/08/81 c33c 4+	No Bleed Reactive for Two or More Antigens	No Bleed Reactive for a Single Antigen	12/5/80 c33c 4+ c22p 2+
DV441119	3/16/81 c33c 2+	No Bleed Reactive for Two or More Antigens	1/20/81 NS5 1+	3/16/81 c33c 3+ NS5 1+
CSH05689	No Bleed Reactive for a Single Antigen	9/14/79 5-1-1 4+ c100-3 2+ c33c 2+	No Bleed Reactive for a Single Antigen	9/14/79 c100p/5-1-1p 1+ c33c 4+
DV91155	12/12/80 c33c 1+	1/15/81 c33c 4+ c22-3 2+	No Bleed Reactive for One Antigen	12/12/80 c33c 4+ c22p 1+
DV061374	10/07/81 c33c 1+	12/15/81 5-1-1 4+ c100-3 3+ c33c 4+	8/05/81 c22p 2+	10/07/81 c33c 4+ c22p 2+

5. Reactivity in High Risk Populations

A performance comparison of the RIBA™ HCV 3.0 and the RIBA™ HCV 2.0 Strip Immunoblot Assays was done using three panels of high risk specimens: 48 Renal Dialysis patients, 49 Intravenous Drug Abusers, and 50 Hemophiliacs. An additional two Renal Dialysis patients were detected as positive by the RIBA™ HCV 3.0 SIA versus the RIBA™ HCV 2.0 SIA. Among Intravenous Drug Abusers, two additional specimens were detected as positive and one additional specimen was detected as indeterminate in the RIBA™ HCV 3.0 SIA versus the RIBA™ HCV 2.0 SIA. Among Hemophiliacs, one additional specimen was detected as indeterminate in the RIBA™ HCV 3.0 SIA. The results are shown in Table 4.

Table 4

PERFORMANCE OF THE RIBA™ HCV 3.0 SIA
IN HIGH RISK PANELS

RENAL DIALYSIS PATIENTS N = 48			
RIBA™ HCV 2.0 SIA		RIBA™ HCV 3.0 SIA	
Positive	1	Positive	3
Indeterminate	3	Indeterminate	1
Negative	44	Negative	44
INTRAVENOUS DRUG ABUSERS N= 49			
RIBA™ HCV 2.0 SIA		RIBA™ HCV 3.0 SIA	
Positive	24	Positive	26
Indeterminate	1	Indeterminate	2
Negative	24	Negative	21
HEMOPHILIACS N = 50			
RIBA™ HCV 2.0 SIA		RIBA™ HCV 3.0 SIA	
Positive	35	Positive	35
Indeterminate	0	Indeterminate	1
Negative	15	Negative	14

6. RIBA™ HCV 2.0 SIA Indeterminate Specimens From High Risk Donors (Hemophiliacs and Renal Dialysis Patients)

The ability of the RIBA™ HCV 3.0 SIA to resolve RIBA™ HCV 2.0 SIA indeterminate results in high risk populations was studied using three panels of RIBA™ HCV 2.0 SIA indeterminate samples. The first panel consisted of 11, c22-3 indeterminate specimens from Hemophiliacs, the second consisted of 37, c22-3 indeterminate specimens from Renal Dialysis patients, and the third contained 5, c33c indeterminate samples from Renal Dialysis patients. Of the 11 RIBA™ HCV 2.0 SIA c22-3 indeterminate specimens from Hemophiliacs, all were resolved as positive by the RIBA™ HCV 3.0 SIA (Table 5). Of the 11 specimens resolved positive, 27.2% were reactive with the c100p/5-1-1p band, 81.8% were reactive with the c33c band and 9.1% were reactive with the NS5 band. Individually, in addition to c22p, 1/11 (9.1%) of the resolved samples were reactive only for the c100p/5-1-1p band, 7/11 (63.6%) of the resolved samples were reactive only for the c33c band, and 1/11 (9.1%) were reactive only for the NS5 band. These data indicate that, in this panel, c100p/5-1-1p, c33c and NS5 all had value in resolving RIBA™ HCV 2.0 SIA indeterminate samples. The c33c band was the key antigen in resolving indeterminate samples because antibody to this antigen was present in 81.8% of the specimens resolved positive and because c33c was the only antigen to resolve 63.6% of the indeterminate samples.

The panel of 37 specimens from RIBA™ HCV 2.0 SIA c22-3 indeterminate renal dialysis patients represented 35 different patients (Two bleeds from each of two patients were included.). Of the 37 specimens, 18 (48.6%) were resolved as positive by the RIBA™ HCV 3.0 SIA, 16 (43.2%) remained indeterminate and 3 samples (8.1%) were resolved as negative (Table 6). In the 18 resolved positive specimens 16.7% were reactive for c100p/5-1-1p, 94.4% were reactive for c33c and 11.1% were reactive for

mac 2/11/99

NS5. In addition, 14 samples (77.8%) were resolved positive by c33c only and 1 sample was resolved positive by NS5 only. No samples were resolved positive only by c100p/5-1-1p. These data again show that c33c is a key antigen in resolving RIBA™ HCV 2.0 SIA indeterminate samples.

Table 5

Resolution of RIBA™ HCV 2.0 SIA Recombinant c22-3 Indeterminate Samples from Hemophiliacs by the RIBA™ HCV 3.0 SIA

(N = 11)

RIBA™ HCV 3.0 SIA RESULTS	NUMBER OF SPECIMENS	PERCENT OF TOTAL
Positive	11	100%
Indeterminate	0	0%
Negative	0	0%

Table 6

Resolution of RIBA™ HCV 2.0 SIA Recombinant c22-3 Indeterminate Samples from Renal Dialysis Patients by the RIBA™ HCV 3.0 SIA

(N = 37)

RIBA™ HCV 3.0 SIA RESULTS	NUMBER OF SPECIMENS	PERCENT OF TOTAL
Positive	18	48.6%
Indeterminate	16	43.2%
Negative	3	8.1%

7. RIBA™ HCV 2.0 SIA Indeterminate Specimens

A total of 107 specimens RIBA™ HCV 2.0 SIA indeterminate for c22-3, 53 specimens RIBA™ HCV 2.0 SIA indeterminate for c33c, and 54 specimens RIBA™ HCV 2.0 SIA indeterminate for c100-3, were tested in the RIBA™ HCV 3.0 SIA with the following results. Of the 107 RIBA™ HCV 2.0 SIA c22-3 indeterminate samples, 37 (34.6%) were resolved as positive by the RIBA™ HCV 3.0 SIA, 21 (19.6%) remained indeterminate, and 49 (45.8%) were resolved as negative. The results are shown in Table 7.

Table 7

Resolution of RIBA™ HCV 2.0 SIA Recombinant c22 Indeterminate Chiron Reference Laboratory Specimens by the RIBA™ HCV 3.0 SIA

(N = 107)

RIBA™ HCV 3.0 SIA RESULTS	NUMBER OF SPECIMENS	PERCENT OF TOTAL
Positive	37	34.6%
Indeterminate	21	19.6%
Negative	49	45.8%

8. Reactivity in Volunteer Blood Donors (Low Risk)

The specificity of the RIBA™ HCV 3.0 SIA was evaluated by testing 324 specimens from presumably healthy volunteer blood donors. Of the 324 donors, no samples were reactive, 8 samples (2.5%) were indeterminate and the rest were negative. A majority of the indeterminate specimens (5/8, 62.5%) showed reactivity toward the c100p band. Reactivity was also seen toward c22p (1/8 or 12.5%) and NS5 (2/8, 25.0%). No indeterminate samples were reactive for c33c. The results are shown in Table 8.

Table 8

RIBA HCV 3.0 SIA Reactivity in
Volunteer Blood Donors (Low Risk)

RIBA HCV 3.0 SIA Result	# (%)
Positive	0 (0.0%)
Indeterminate	8 (2.5%)
Negative	316 (97.5%)
Total Number Tested:	324 (100.0%)

V. CLINICAL TRIALS DATA

A. Clinical Trial Summary

The performance of the CHIRON® RIBA™ HCV 3.0 SIA was evaluated in clinical studies of low risk, high risk, and NANBH populations. Specificity was evaluated in three populations:

(1) serum specimens from normal volunteer blood donors, collected prospectively and tested at three blood centers; (2) archival specimens from deferred blood donors who were repeatedly reactive by a licensed multi-antigen screening assay; (3) specimens from individuals with liver diseases other than non-A non-B Hepatitis (NANBH). Sensitivity was evaluated using serially collected specimens from NANBH patients (seroconversion panels); specimens from patients with clinically documented NANBH; and specimens from persons at high risk for acquiring NANBH, such as hemophiliacs, long-term hemodialysis patients, and intravenous drug users (IVDU). All specimens were tested in parallel by the CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA.

B. Reactivity in Volunteer Random Blood Donors (Low Risk)

A total of 3004 sequential, previously unscreened, random blood donations were collected prospectively and tested at three blood centers participating as clinical sites. Table 9 summarizes the results. Of these 3004 donors, 5 were positive by both assays and 2955 were negative by both assays. Twenty-nine specimens were indeterminate by CHIRON® RIBA™ HCV 3.0 SIA only and 9 were indeterminate by CHIRON® RIBA™ HCV 2.0 SIA only. Assuming that these indeterminate results represent uninfected individuals, the specificity of CHIRON® RIBA™ HCV 2.0 SIA was 99.5% (2984/2999) and the specificity of CHIRON® RIBA™ HCV 3.0 SIA was 98.8% (2964/2999). (Specificity was calculated as follows: TN/(TN + FP), where TN = true negatives, that is, the number of specimens negative by CHIRON® RIBA™ SIA; and FP = false

positives, that is , the number of specimens indeterminate by CHIRON® RIBA™ SIA.

Table 9
CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA
Testing Results for Volunteer Blood Donors from Three Blood Centers
(Low Risk)

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	5	0	0	5 (0.2%)
Indeterminate	0	6	29	35 (1.1%)
Negative	0	9	2955	2964 (98.7%)
TOTAL	5 (0.2%)	15 (0.5%)	2984 (99.3%)	3004 (100%)

C. Reactivity in Volunteer Blood Donor Specimens Repeatedly Reactive by a Licensed Multi-Antigen Anti-HCV Screening Procedure

In two separate studies, the CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA were evaluated with a total of 851 specimens which were repeatedly reactive by licensed Version 2.0 or Version 3.0 multi-antigen anti-HCV screening procedures.

In the first study, a total of 732 specimens that were repeatedly reactive by licensed Version 2.0 multi-antigen screening procedures were tested with the CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA. The results of these studies are shown in Table 10. Of these 732 specimens, a larger number were negative by CHIRON® RIBA™ HCV 3.0 SIA than by CHIRON® RIBA™ HCV 2.0 SIA, and a smaller number were found to be indeterminate by CHIRON® RIBA™ HCV 3.0 SIA than by CHIRON® RIBA™ HCV 2.0 SIA. Therefore, CHIRON® RIBA™ HCV 3.0 SIA provides a more definitive indication of the presence or absence of HCV antibody.

Table 10

Comparison of CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA on Specimens Repeatedly Reactive by a Licensed Version 2.0 Multi-antigen Anti-HCV Screening Procedure

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	432	26	2	460 (62.8%)
Indeterminate	2	34	18	54 (7.4%)
Negative	2	165	51	218 (29.8%)
TOTAL	436 (59.6%)	225 (30.7%)	71 (9.7%)	732 (100%)

In the second study, a total of 119 specimens which were repeatedly reactive by a licensed Version 3.0 multi-antigen screening procedure were tested with the CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA. These 119 specimens represent an unscreened donor population of approximately 30,000 individuals. The results of this study are given in Table 11. Of these specimens, a greater number were found to be either positive or indeterminate by CHIRON® RIBA™ HCV 3.0 SIA than by CHIRON® RIBA™ HCV 2.0 SIA. Therefore, CHIRON® RIBA™ HCV 3.0 SIA demonstrates increased sensitivity on EIA 3.0 repeatedly reactive samples compared to CHIRON® RIBA™ HCV 2.0 SIA.

Table 11

Comparison of CHIRON® RIBA™ HCV 3.0 SIA and CHIRON® RIBA™ HCV 2.0 SIA on Specimens Repeatedly Reactive by a Licensed Version 3.0 Multi-antigen Anti-HCV Screening Procedure

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	78	3	0	81 (68.1%)
Indeterminate	0	11	12	23 (19.3%)
Negative	0	3	12	15 (12.6%)
TOTAL	78 (65.5%)	17 (14.3%)	24 (20.2%)	119 (100%)

D. Specificity in Specimens from Individuals with Other Liver Diseases or Elevated Levels of Immunoglobulins

Two study sites tested a total of 364 specimens from persons with initial diagnoses of liver diseases other than NANBH. These included HAV, acute and chronic HBV, CMV, autoimmune hepatitis, nonviral liver disease, and specimens with elevated IgA, IgM, or IgG. Results are presented in Table 12. There were only three specimens with discrepant results between CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA. Two of these specimens were negative by a licensed multi-antigen anti-HCV screening procedure; the other was repeatedly reactive.

Table 12

CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA Results on Specimens from Subjects with Other Liver Diseases

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	9	0	0	9 (2.5%)
Indeterminate	0	3	3	6 (1.7%)
Negative	0	0	349	349 (95.8%)
TOTAL	9 (2.5%)	3 (0.8%)	352 (96.7%)	364 (100%)

E. Sensitivity in Seroconversion Panels

A total of 86 seroconversion panels from individuals with documented seroconversion to antibodies to HCV and clinical documentation of NANBH were tested. Subjects were categorized as acute if two sequential serum specimens had SGPT/ALT levels greater than 44 IU/L and 90 IU/L, respectively, and the SGPT/ALT level returned to normal within six months. Subjects whose SGPT/ALT level remained greater than two times the upper limit of normal for longer than six months were categorized as having chronic NANBH. All subjects diagnosed as having NANBH were serologically negative for hepatitis A and hepatitis B.

Table 13 summarizes the CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA results from these panels.

Table 13

Summary of Seroconversion Panel Testing Results

Category of NANBH	N	Equal Sensitivity*/Equal Reactivity*	EqualSensitivity/CHIRON® RIBA™ HCV 3.0 SIA Greater Reactivity	CHIRON® RIBA™ HCV 3.0 SIA Greater Sensitivity
Acute	32	13	9	10
Chronic	41	14	10	17
Indeterminate	9	3	2	4
Not Specified	4	4	0	0
TOTAL	86 (100%)	34 (40%)	21 (24%)	31 (36%)

* The term "sensitivity" refers to any reactivity (indeterminate or positive) vs no reactivity; "reactivity" refers to the magnitude of reactivity (indeterminate vs positive).

In 52 of the 86 panels (60%), CHIRON® RIBA™ HCV 3.0 SIA showed greater reactivity or sensitivity than CHIRON® RIBA™ HCV 2.0 SIA. In 21 panels (24%), both assays detected anti-HCV on the same blood draw, but CHIRON® RIBA™ HCV 3.0 SIA became positive earlier than CHIRON® RIBA™ HCV 2.0 SIA (i.e., greater reactivity). In 31 panels (36%), CHIRON® RIBA™ HCV 3.0 SIA detected anti-HCV earlier than CHIRON® RIBA™ HCV 2.0 SIA, with a mean difference of 50 days (range 9 to 282 days) between detection by CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA. In no case, was CHIRON® RIBA™ HCV 3.0 SIA less sensitive/reactive than CHIRON® RIBA™ HCV 2.0 SIA.

F. Sensitivity for Patients with Acute and Chronic NANBH

A total of 239 specimens from patients with documented acute NANBH and 96 specimens from patients with documented chronic NANBH were evaluated.

The summary results from testing the acute NANBH specimens are shown in Table 14. These subjects had the following clinical findings: a serum SGPT/ALT level of greater than 500 IU/L (site 1) or 1000 IU/L (site 2); no reported use of hepatotoxins; and negative serology for anti-HAV IgM, anti-HBc IgM, and

HBsAg. A greater number of the acute NANBH specimens were reactive (i.e., positive or indeterminate) by CHIRON® RIBA™ HCV 3.0 SIA than by CHIRON® RIBA™ HCV 2.0 SIA (187 and 174, respectively), and CHIRON® RIBA™ HCV 3.0 SIA provided a more definitive indication of the presence or absence of HCV antibody (positive or negative vs indeterminate) than CHIRON® RIBA™ HCV 2.0 SIA. Of the 173 specimens that were reactive by both assays, CHIRON® RIBA™ HCV 3.0 SIA was positive for 154 (89%) compared to 132 (76%) for CHIRON® RIBA™ HCV 2.0 SIA.

Table 14

CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA
Results on Specimens from Patients with Acute NANBH

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	131	23	2	156 (65.2%)
Indeterminate	1	18	12	31 (13.0 %)
Negative	0	1	51	52 (21.8%)
TOTAL	132 (55.2%)	42 (17.6 %)	65 (27.2 %)	239 (100%)

Table 15 presents the summary testing results on 96 specimens from patients with documented chronic NANBH. These subjects demonstrated persistently elevated SGPT/ALT levels (greater than two times the upper level of normal) for more than six months, no history of hepatotoxin abuse, and were negative for HBsAg. The results showed no differences in sensitivity or reactivity between CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA in patients with chronic NANBH.

Table 15

CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA Results on Specimens from Chronic NANBH Patients

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	83	0	0	83 (86.5%)
Indeterminate	0	1	0	1 (1.0%)
Negative	0	0	12	12 (12.5%)
TOTAL	83 (86.5%)	1 (1.0%)	12 (12.5%)	96 (100%)

G. Sensitivity in High Risk Populations

The performance of the CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA was evaluated with a total of 614 specimens collected from members of high risk groups, comprising hemophiliac patients, hemodialysis patients, and intravenous drug abusers. The results are shown in Table 16. A greater number of these specimens were reactive by CHIRON® RIBA™ HCV 3.0 SIA than by CHIRON® RIBA™ HCV 2.0 SIA (417 vs 408, respectively). As in the acute NANBH population, a higher percent of the specimens that were reactive by both assays were positive by CHIRON® RIBA™ HCV 3.0 SIA (97.5%, 397/407) than by CHIRON® RIBA™ HCV 2.0 SIA (94.3%, 384/407).

Table 16

CHIRON® RIBA™ HCV 2.0 SIA and CHIRON® RIBA™ HCV 3.0 SIA Results in High Risk Populations

CHIRON® RIBA™ HCV 3.0 SIA	CHIRON® RIBA™ HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	383	14	5	402 (65.5%)
Indeterminate	1	9	5	15 (2.4%)
Negative	0	1	196	197 (32.1%)
TOTAL	384 (62.5%)	24 (3.9%)	206 (33.6%)	614 (100%)

H. Specific Performance Characteristics

1. Potentially Interfering Substances and Conditions

The effect of elevated levels of triglycerides, bilirubin, and hemoglobin were evaluated in the CHIRON® RIBA™ HCV 3.0 SIA using anti-HCV positive and anti-HCV negative specimens. The effect of microbial contamination was also evaluated, using specimens spiked to a final concentration of 103 CFU/mL with *Candida albicans*, *Pseudomonas aeruginosa*, or *Staphylococcus epidermidis*. The microbially contaminated specimens were tested on the day of spiking, at Day 3, and again at Day 8. The effect of multiple freeze-thaws and heat inactivation were also evaluated. The results are presented in Table 17. Assay results were comparable under all conditions tested.

Table 17
Effect of Potentially Interfering Substances and Conditions on
CHIRON® RIBA™ HCV 3.0 SIA

Substance or Condition	Level of Interferent or Description of Condition	Effect
Triglycerides	up to 1600 mg/dL	No Effect
Bilirubin	up to 60 mg/dL	No Effect
Hemoglobin	up to 80 mg/dL	No Effect
Microbial contamination	10^3 for up to 8 days	No Effect
Freeze-thaws	5 cycles	No Effect
Heat inactivation	56¼C for 1 hour	No Effect
Anti-yeast antibodies	4 specimens	No Effect
Anti-E. coli antibodies	22 specimens	No Effect

2. Specimen Collection Devices

The performance of the CHIRON® RIBA™ HCV 3.0 SIA was evaluated in anti-HCV positive and anti-HCV negative specimens collected in serum Vacutainer tubes, serum separator tubes, and in the following anticoagulants: K3 EDTA (15% solution), ACD (Solution B), sodium heparin, CPDA-1, and 4% sodium citrate. An additional study compared

the pilot (serum) tube specimen to the specimen from the blood bag segment. Assay results were comparable with all specimen collection devices and anticoagulants tested.

3. Analytical Sensitivity

A dilutional analysis of 10 anti-HCV positive specimens was performed comparing results from the CHIRON® RIBA™ HCV 3.0 SIA and the CHIRON® RIBA™ HCV 2.0 SIA. Individual antigen band results were evaluated to determine the lowest detectable dilution for each band for each test. For each antigen band of each specimen, the CHIRON® RIBA™ HCV 3.0 SIA had the same or greater dilutional sensitivity as the CHIRON® RIBA™ HCV 2.0 SIA. The percent of antigen band determinations with greater dilutional sensitivity using the CHIRON® RIBA™ HCV 3.0 SIA was 40% for the c22 band, 50% for the c100 band, and 80% for the c33c band. In no case was the dilutional sensitivity of CHIRON® RIBA™ HCV 3.0 SIA less than that of CHIRON® RIBA™ HCV 2.0 SIA.

4. Reproducibility

The precision of the CHIRON® RIBA™ HCV 3.0 SIA was established in two studies, one that assessed assay reproducibility across assay runs, operators, sites, and lots; the second that assessed the reproducibility of antigen band and strip interpretations under different lighting conditions. Both studies utilized the same six-member panel, which included three positive specimens, two indeterminate specimens, and one negative specimen.

In the first study, three operators at each of three sites performed the testing. Each operator tested the panel in singlicate in three different runs using each of three kit lots. Therefore, for each of the three kit lots, there were a total of 27 CHIRON® RIBA™ HCV 3.0 SIA strips tested with

each panel member. In the study, no strips were incorrectly interpreted. In addition, 492 of 500 (98.4%) antigen band ratings were within one level of intensity of the consensus result. Five of the 8 discordant ratings were +/- when the consensus reading was 2+. The other three cases involved band ratings of 4+ when the consensus reading was 2+. The majority of the variability in band interpretation was attributable to within-run and between-run components. These data demonstrate that the scoring of the intensity of CHIRON® RIBA™ HCV 3.0 SIA antigen bands is reproducible across multiple sites, operators, and lots.

At one site, the reproducibility panel was tested to assess the effect of different lighting conditions (fluorescence, incandescence, or natural lighting) on interpretation of the CHIRON® RIBA™ HCV 3.0 SIA strips. Each panel member was read a total of 27 times under each of the three lighting conditions, for a total of 81 readings. Regardless of lighting condition, all panel members were interpreted correctly by all readers with all kit lots. The interpretation of individual antigen bands for each panel member was also consistent from one lighting condition to another. The data demonstrated that scoring of intensity of the CHIRON® RIBA™ HCV 3.0 SIA was not affected by lighting conditions.

VI. PACKAGE INSERT

A copy of the package insert (directions for use) is attached.

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