REPORT FOR THE DIRECTOR ON
2nd INTERNATIONAL SYMPOSIUM ON
HEPATITIS C VIRUS

Los Angeles, November 1990

J Gillon

HOUGHTON

Nucleocapsid proteins highly conserved. Envelope proteins much more variable. US isolates show strong homology (> 90%) but there are 2 Japanese isolates showing only ~70% homology with US, 80% with each other.

KUO

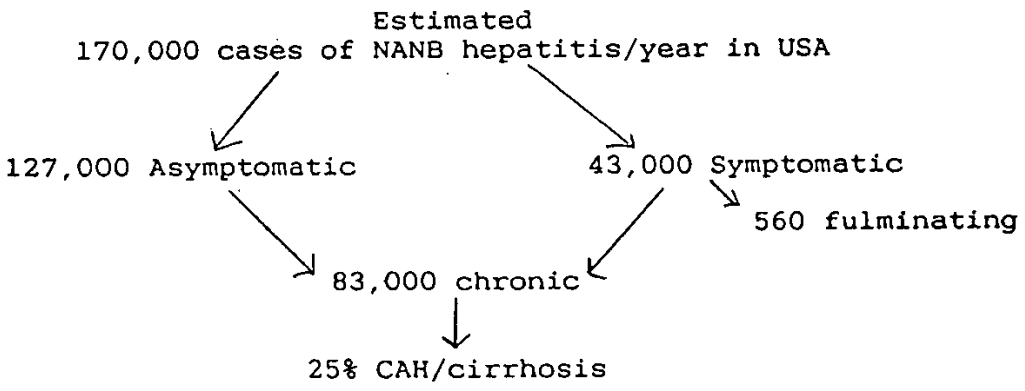
"True" C-100 pos serum is 80% infectious, though not all seroconvert. C33c Ag picks up seroconversion much earlier. It has a higher % pick-up rate on acute and chronic NANB hepatitis. C22 Ag also useful. Second generation will have C-100, C33c and C22. All 3 show good correlation with PCR, but there are some paid donors (Miami) with full house of Ab's, including to other Ags, who are PCR negative. Not known whether they are infectious.

PURCELL (USA)

Nested PCR reliably detects 10^{-7} dilution of infectious serum, when chimp infectivity lost at 10^{-6}. 5'9 primers are the most reliable currently available. Occassionally negative PCR's seen during course of infection. PCR (in chimps) can be very transient even in low risk hepatitis, preceding ALT rise, with weak anti C-100. Some such cases may be diagnostic problems - will need more refined tests such as the previous paper, or in-situ hybridation etc.

Anti-S_2 may have potential as hyperimmune globulin.

M ALTER



Only 5% of acute NANB in USA in 1989 reported transfusion in previous 6 months. IVDA 35% 43% no identifiable source. This 5% represents a significant recent decline. This started at time of HIV exclusion, and rate of decline was not affected by introduction of surrogate screening.

Progression to chronicity same regardless of aetiology.

Good correlation between EIA and RIBA(1) in high risk individuals, including implicated donors (> 80%), while < 30% in non-implicated donors.

Random digit dialling -> 2-3% seropositivity but only 20% of these thought to be true positive.

No evidence of insect vector.

Sexual Transmission

Two case control studies suggest low socioeconomic status, sexual or household contacts with previous NANB contacts, or multiple sex partners -> high prevalence. Seroprevalence studies also suggest that this is an important route of spread, eg 20% of female partners of IVDA's.

But there are also studies failing to show important effect of sex or household contacts, and there are plenty of studies showing much lower anti-HCV than anti HBc and HIV in high risk populations.

A lot of this probably reflects insensitivity of tests.

H ALTER

Cirrhosis rate in his cohort now up to 20% (8 patients - 7 HCV positive). Two early decompensation, 4 out of the 8 doing well. Overall in their 40 patients:-

30% improved 45% stable 25% progressed

There are now a few well documented (about 6) cases from other studies going acute icteric heaptitis -> CPH -> CAH -> cirrhosis -> HCC. All HCV ab positive.

Barcelona studies: no effect of surrogate testing on NANB post transfusion hepatitis. Rate fell on anti-HCV screening from 9.6% to 2.8%. 84% anti HCV positive donors transmitted (comparable figure from NIH).

anti HCV and raised ALT -> 100% infective
anti HCV and 5-1-1 -> 97% infective
anti HCV and liver histol -> 92% infective

RIBA-2 = C33c and C22 as well as C-100 and 5-1-1. Patterns vary but overall more sensitive than EIA or RIBA-1.

Cautious against interpreting early RIBA-2 in PTH cases = passive Ab easily detectable up to weeks 6-8. Using C33c all seroconversions occur within 20 weeks, cf up to 1 year with c-100. 89% of NANB PTH cases have RIBA-2 positive donor, cf 80% for EIA.

Both Alters very cool about NHI in needlestick prophylaxis - "current recommendations shouldn't be changed", ie 0.6 ml/Kg may be given when high risk of transmission.

ESTEBAN-MUR (Barcelona)

2.4% PTH in spite of anti-HCV screening, i.e. transmission by anti-HCV negative, normal ALT donors. Most of these recipients seroconvert.

Most of the HCV negative, normal ALT donors who transmit do not subsequently seroconvert - ie they are chronic carriers negative for markers currently not detectable.

83 anti-HCV positive donors biopsied. Those with high OD mostly had some abnormality, and even in those with low OD and normal ALT, > 50% had some abnormality. Chronic hepatitis in 75%.

Older age at onset, long duration and HIV positivity associated with worse histology - also alcohol. Possibly HCV makes chronic hepatitis B worse and vice-versa.

No difference in outcome between transfusion associated and sporadic HCV - 8 years follow-up (n = 75 versus 214) cirrhosis 21% and 32% response overall 4% died.

GOOD		BAD
Young	Age at infection	Old
Short	Duration	Long
Normal	ALT	Raised
Normal	Immune status	Impaired
Early decline	HCV Ab	Persistent
Mch/CPH	Histology	CPH/Cirrhosis
None	Alcohol/HBV cofactors	+/+

Estimate 2 x 10^6 (0.7%) carriers in Western Europe - how is this useful?

BONINO (Italy)

Steatosis common in HCV CAH - 65%. Suggested that steatosis may be pre-existent ie it predisposes to chronicity of HCV.

Decline in titre of anti-33c is seen in all IFN responders - probably best epitope for monitoring therapy.

NOEL (France)

22% only of donors with surrogate markers are HCV positive.

57.3% of HCV positive donor had risk factors

46% abroad (3rd World countries)

30% transfusions

15% HCW

12% IVDA

Hospital without transfusion 7.5% (MANY related to ENDOSCOPY)

Risk of HCV/1000 units lowered from 4.3 to 1.9 after surrogate screening.

Clear relationship between OD and EIA and positivity on RIBA-1.

191 HCV positive donors followed at Hospital St Antone:-

30% raised ALT x 2 n

- history of transfusion
- IVDA
- raised OD
- positive RIBA-1
- > 50% CAH on biopsy*

P HOLLAND (California)

> 50% of donors with both surrogate markers had HCV (cf 22% in French studies). This is similar to Moseley's figures, and he showed that 75% were infectious.

Advice to HCV positive donors:-

	True +	False +
Stop giving	yes	yes
referral	yes	no
precautions	yes (but not condoms with regular partners. No alcohol).	no
reinstatement	doubt hope	

NIH has telephone advice line for HCV!

5-6% of donors in USA have history of transfusion. In Sacramento none of these has been HCV positive, therefore, no indication for exclusion on this basis.

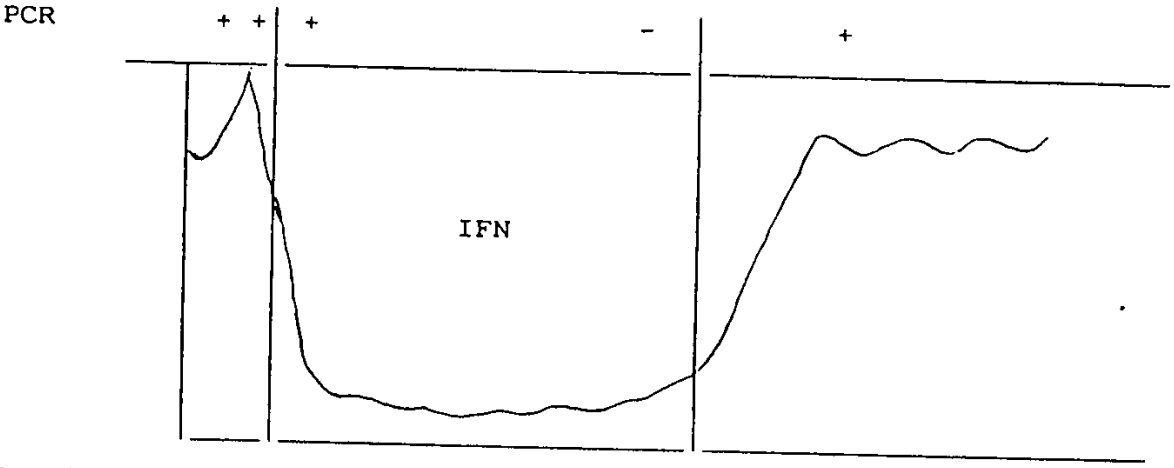
Note 2 important side effects of IFN:-

1 Raised susceptibility to bacterial infection
2 induction of autoimmunity - most often thyroid, but also ITP, AIHA, SLE etc.

J HOOFNAGLE

Cannot predict responders to IFN/

PCR goes negative with normalisation of ALT, but negative PCR at end of Rx does not predict absence of relapse. This can happen:-



Acyclovir no effect
Ribavirin may have a role at 1000 ug/day.

α - IFN in chronic Hepatitis C

- Initial exacerbation not seen
- Autoimmune hepatitis gets worse
- WBC + platelets ↓, sometimes quite low, but bleeding very rare. Reduce IFN by 50% - don't stop.
- After cessation, transient flare-ups common, often requiring no Rx. Re-treat persistent relapse.

Blood Banking Concensus

Notify and exlude all HCV positive donors (EIA only)
Surrogate testing to continue in USA
Auto transfusion mandatory
Lookback unresolved

1 Poster E28

BMT patients - no effect on outcome (cf renal Tx) ie no evidence that the immunosuppression make it progress.

2 Poster E40

Transmission of HCV and HBV in contacts of haemophiliacs.

	% HBsAg * or anti-HBc	% anti HCV
SEXUAL	18.2	2.5
Other Contacts	33.3	2.32

* probands chronic HBsAg carriers.

3 Poster E20

Very reassuring data on vertical transmissions - generally endorsed. But conflicting evidence from San Francisco. PCR shows babies get virus, but don't get trouble unless also HIV positive.

4 Poster E24

Reassuring data on needlesticks. Schiff and Desmyter confirmed absence of anti-HCV in personnel working in dialysis units.

APPENDIX 1

NATIONAL HEPATITIS DETECTION AND TREATMENT PROGRAM

EDUCATIONAL COUNCIL GOALS

To disseminate knowledge of epidemiology and natural history of hepatitis C.

- Provide medical education to physicians and other healthcare practitioners in order to decrease incidence, morbidity, and mortality resulting from HCV infection.
- Provide a forum for analysis of scientific and clinical advances related to HCV.
- Raise priority for identification and follow-up of patients with HCV infection.
- Provide multidisciplinary insight into the policies and procedures being developed regarding identification and prevention of HCV infection.

To determine the appropriate uses and recognized limitations of HCV testing.

- Establish proper patient criteria for ELISA anti-HCV screening and utilization of supplemental tests.
- Provide direction to clinicians concerning the issue of false-positive and false-negative test results.
- Via a testing algorithm, facilitate the proper use of HCV testing by clinicians.
- Encourage third-party and managed healthcare coverage of HCV testing by providing an understanding of its benefits.

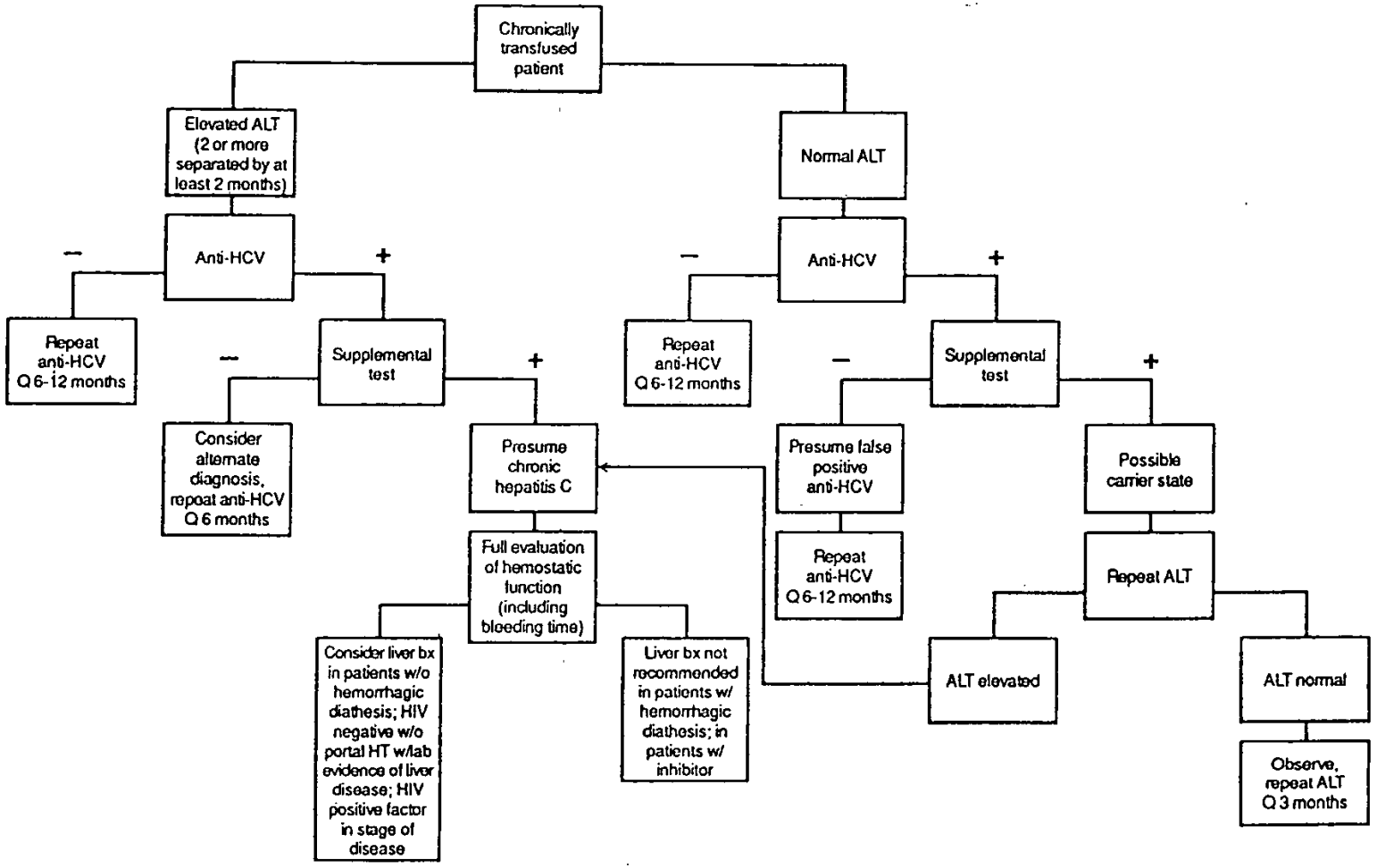
To develop guidelines for the treatment and follow-up management of patients with diseases related to HCV.

- Establish guidelines for the initiation of therapy with interferon, develop knowledge regarding the evaluation of response, and determine therapeutic end points.
- Via a treatment algorithm, identify the progression and stages of HCV infection and the appropriate therapeutic regimen.
- Disseminate information regarding side effects and develop methods to increase compliance with interferon therapy.
- Establish appropriate treatment guidelines for follow-up during and after treatment.

EDUCATIONAL COUNCIL GOALS (cont.)

To develop consensus among medical practitioners regarding the diagnosis and treatment of HCV infection and the means to disseminate information to practicing physicians.

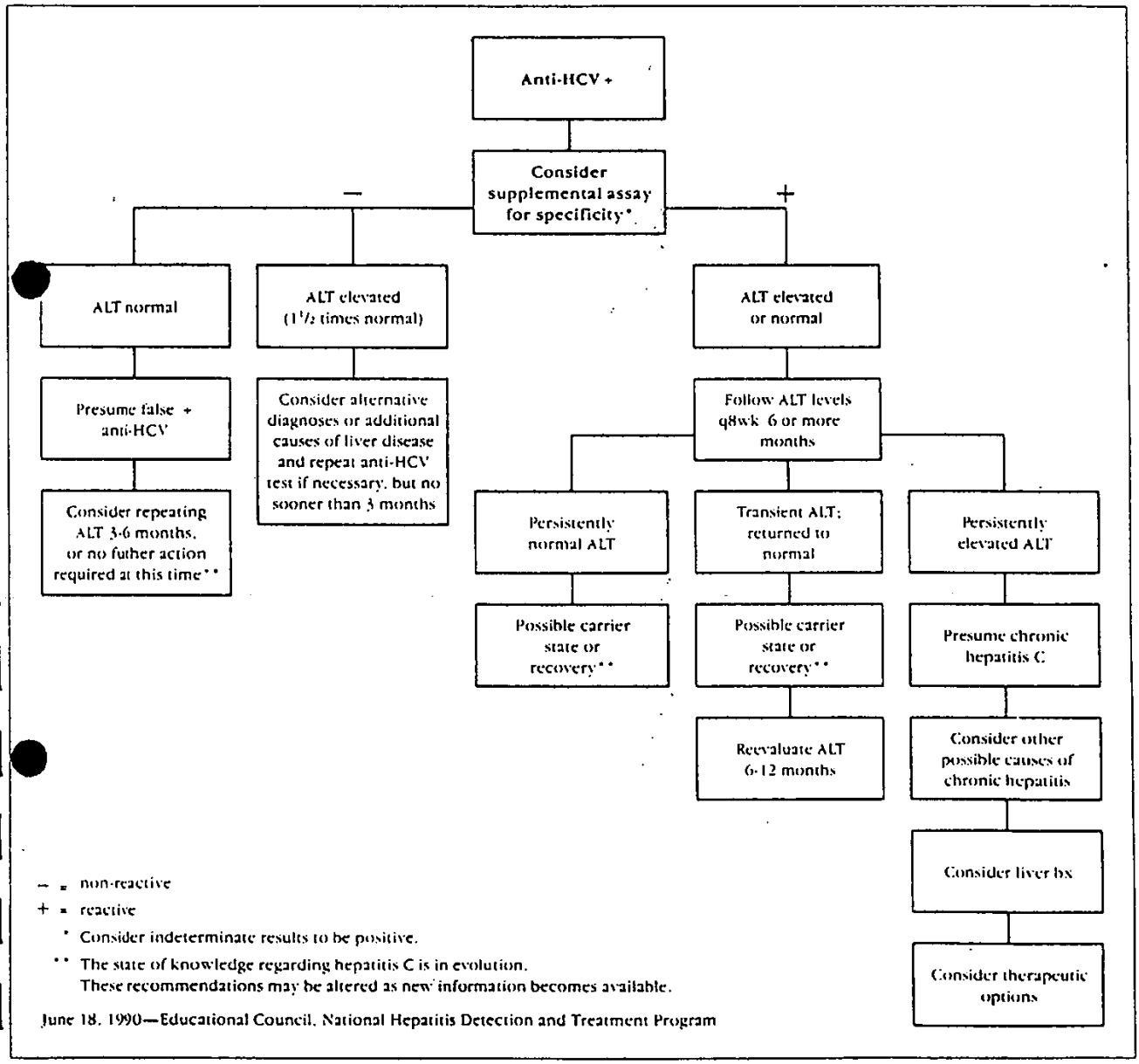
- Create an open forum for the exchange of information among highly informed scientists and physicians.
- Translate scientific advances into practical information that can be utilized in daily practice.
- Disseminate the consensus findings to relevant practitioners in a variety of settings in order to foster improved patient care.
- Facilitate the cooperative interaction between specialist and generalist to enhance patient care.
- Assist physicians in counseling hepatitis C patients.
- Promote the development of outcome assessment programs.



*The state of knowledge regarding hepatitis C is in evolution.
These recommendations may be altered as new information becomes available.

June 8, 1990 — Hematology Caucus, National Hepatitis Detection and Treatment Program

HCV Infection — An Approach to Evaluation of Anti-HCV + Patient



APPENDIX 2

B29. Improved Detection of Antibodies to Hepatitis C Virus in Cases of Non-A, Non-B Hepatitis Using A Second Generation (c200/c22) ELISA

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[2]Division of GI and Liver Diseases, University of Southern California, Los Angeles, California
[3]Chiron Corporation, Emeryville, California, USA

INTRODUCTION

Hepatitis C virus (HCV) is now known to be the major etiologic agent of parenterally transmitted non-A, non-B (NANB) hepatitis. The ORTHO™ HCV ELISA Test System has been shown to be an effective methodology for the detection of circulating antibodies to recombinant HCV c100-3 antigen. We now report the development of a second generation ELISA that detects antibodies to structural (c22-3) and non-structural (c200) HCV proteins. We have assessed the sensitivity and specificity of this assay or the detection of anti-HCV in cases of acute and chronic NANB hepatitis.

METHODS

Specimens from patients diagnosed with NANB hepatitis were tested for anti-HCV using both first generation (c-100-3) and second generation (c-200/c22-3) ELISA test systems. The second generation ELISA differs from the current (c-100-3) assay in that it detects antibodies to both non-structural (c-200) and structural (c22-3) proteins.

Criteria for diagnosis of ACUTE NANB included:

1. Peak ALT > 1000 IU/ml.
2. Peak bilirubin > 50umol/L.
3. HBsAg negative.
4. Anti-HBcAb IgM negative.
5. Anti-HAV IgM negative.

Sera from CHRONIC NANB Hepatitis was characterized by:

1. HBsAg negative.
2. At least two samples with ALT >2x normal at least 6 months apart with no other explanation apparent.
3. No episode of acute hepatitis within 6 months of bleed date.

In addition to testing by ELISA, specimens were also tested by second generation RIBA™ HCV Test System from Chiron® Corporation. This test is a recombinant immunoblot assay capable of detecting antibodies to HCV antigens 5-1-1, c100-3, c33c and c22-3.

REACTIVITY OF SPECIMENS FROM NANB HEPATITIS PATIENTS IN CHIRON® RIBA™ HCV TEST SYSTEM

DISEASE PHASE	2ND GEN ELISA RESULT	RIBA RESULT 5-1-1	c100-3	c33c	c22-3	INTERPRETATION
A	N	+/–	+/–	+/–	+/–	NEG
A	R	3+	+/–	2+	2+	POS
A	R	+/–	2+	2+	4+	POS
A	R	+/–	–	4+	4+	POS
A	R	–	+/–	–	4+	IND
A	R	4+	4+	4+	4+	POS
A	N	–	–	–	+/–	NEG
A	R	+/–	4–	+/–	4+	POS
A	R	4+	4+	4+	+/–	POS
A	R	1+	+/–	3+	4+	POS
C	N	+/–	+/–	+/–	+/–	NEG
C	R	4+	4+	4+	4+	POS
C	R	4+	4+	4+	4+	POS
C	N	–	–	–	+/–	NEG
C	N	–	–	–	+/–	NEG
C	R	4+	2+	2+	4+	POS
C	R	4+	4+	4+	4+	POS
C	R	+/–	+/–	4+	4+	POS
C	R	2+	2+	4+	4+	POS
C	R	–	4–	2+	4+	POS
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C	R	4+	4+	4+	4+	POS
C	R	+/–	+/–	4+	4+	POS
C	R	4+	4+	4+	4+	POS
C	R	2+	+/–	4+	2+	POS
C	R	4+	4+	4+	4+	POS
C	R	–	–	4+	+/–	IND

A = Acute NANB
C = Chronic NANB
R = Reactive
N = Nonreactive

Figure 2

SPECIMENS REACTIVE IN SECOND GENERATION ELISA (c200/c22-3) ONLY

Specimen ID #	Diagnosis	ELISA OD c100-3	ELISA OD c200/c22-3	ALT	RIBA Reactivity
4	ACUTE	0.070	2.500	319	C33C, C22-3
5	ACUTE	0.181	2.500	280	C22-3
18	CHRONIC	0.417	2.500	48	C33, C22-3
27	CHRONIC	0.204	2.500	58	C22-3
35	CHRONIC	0.285	2.500	65	C33C

Figure 3

ASSOCIATION OF RISK FACTOR WITH HCV ELISA REACTIVITY IN CASES OF NANB HEPATITIS

Risk Factor	No. of Specimens	ELISA REACTIVITY 1ST and 2ND	1ST only	2ND only	Negative
Post - Transfusion	17	15	0	2	0
IV Drug Use	9	7	0	2	0
Needle - Stick	1	0	0	1	0
Unknown	8	3	0	0	5

Figure 4

PREVALENCE OF SPECIMENS REACTIVE IN 1ST AND 2ND GENERATION ELISA FROM PATIENTS WITH CHRONIC AND ACUTE NANB HEPATITIS

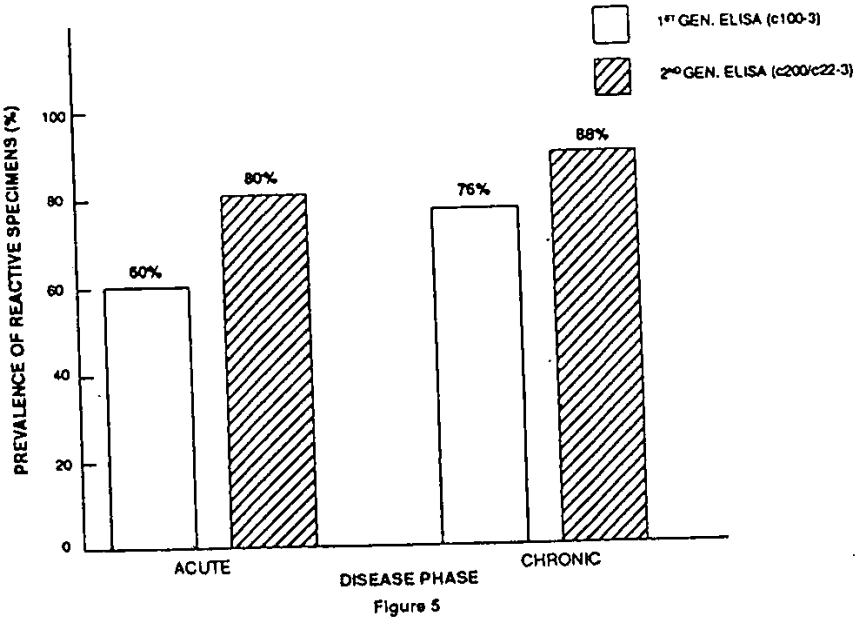


Figure 5

CONCLUSIONS

1. ORTHO™ first and second generation ELISA test systems demonstrate a very high concordance with Chiron® RIBA for the detection of HCV Antibodies in cases of acute and chronic hepatitis.

2. ORTHO™ second generation ELISA (c200/c22-3) detected a significantly greater number of specimens with antibodies to HCV than first generation ELISA (c100-3).

3. Additional specimens that were reactive only in second generation ELISA, demonstrated reactivity to HCV antigens when tested in RIBA.

4. ORTHO second generation ELISA (c200/c22-3) provides improved sensitivity for the detection of antibodies to HCV in cases of parenterally transmitted NANB hepatitis.

APPENDIX 3

A SECOND GENERATION ELISA (c200/c22) FOR THE DETECTION OF ANTIBODY TO HEPATITIS C VIRUS

S. Lee[1], B. Francis[1], V. Ball[1], R. Kochesky[1], E. Page[1], S. Quan[2], A. Polito[2], M. Houghton[2], G. Kuo[2] and M. Nelles[1].

[1]Ortho Diagnostic Systems Inc., Raritan, NJ [2]Chiron Corp. Emeryville, CA.

INTRODUCTION

The ORTHO™HCV ELISA Test System has been shown to be an effective methodology for the detection of antibodies to hepatitis C virus (HCV). We now report the development of a second generation ELISA with improved clinical performance for the detection of anti-HCV antibodies. This assay detects antibodies to both structural (c22-3) and non-structural (c200) HCV proteins. We have assessed the sensitivity and specificity of this assay for the detection of anti-HCV in a variety of clinical populations.

METHODS

Specimens derived from various clinical groups were tested for the presence of HCV antibodies using the current ORTHO ELISA (c100-3) and by second generation ELISA (c200/c22-3). Approximately 500 specimens from clinical populations generally considered to be at high risk for HCV infection were tested in addition to approximately 2000 normal blood donors. Initially reactive specimens were retested in duplicate. Discordant specimens from the high risk groups and all repeat reactives from the normal donors were confirmed using a second generation RIBA™ HCV Test System from Chiron Corp., which is recombinant immunoblot assay capable of detecting antibodies to recombinant HCV proteins 5-1-1, c100-3, c33c and c22-3. In this study, samples tested in RIBA were considered positive if they displayed reactivity to two or more antigen bands and indeterminant if they displayed single band reactivity.

RESULTS

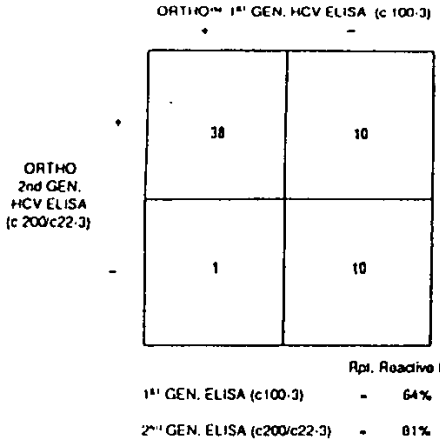
In each of the high risk groups studied, the second generation ELISA (c200/c22-3) showed good concordance with the first generation (c100-3) assay. However, in each clinical population, the rate of repeat reactivity was higher in the case of the second generation ELISA (Figures 1-6). When specimens reactive only in second generation ELISA were tested by RIBA, most were positive. A smaller proportion displayed reactivity to a single HCV antigen (indeterminant). A small number of discordants that were reactive only in first generation ELISA or only in second generation ELISA were all negative when tested in RIBA. These results indicate that the second generation (c200/c22-3) ELISA provides improved sensitivity for the detection of anti-HCV antibody in high risk groups.

A total of 1992 serum specimens were tested by both first and second generation ELISA. First generation ELISA demonstrated an initial reactive rate of 1.20% and a repeat reactive rate of 1.05%. Second generation ELISA demonstrated an initial reactive rate of 1.05% and a repeat reactive rate of 1.00% (Figure 9). Of the 21 specimens repeatably reactive in first generation ELISA, 7 (33%) were positive when tested by RIBA. By contrast, 12 (60%) of the 20 repeat reactives in second generation ELISA were positive in RIBA. These data indicate that second generation ELISA would detect at least an additional 2 true positives per 1000 in a presumably healthy volunteer donor population. Eleven of the first generation reactives were negative in RIBA, while only 3 of the second generation reactives were RIBA negative. The apparent false-positivity rate of first generation ELISA was 0.55%. The rate of false-positivity in second generation ELISA was significantly lower at 0.15%.

HEMOPHILLIACS
(N = 58)

ELISA RESULTS

RIBA™ ANALYSIS OF DISCORDANTS



ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	10*	7	2	0
1.0 ONLY	1	0	0	1

*1 = ONS

Rpt. Reactive Rate

1st GEN. ELISA (c100-3) - 64%

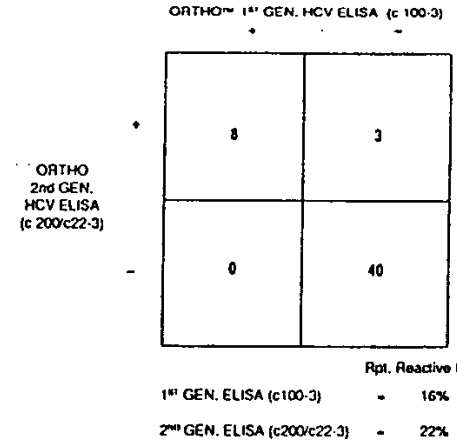
2nd GEN. ELISA (c200/c22-3) - 81%

Figure 1

HEMODIALYSIS PATIENTS
(N = 51)

ELISA RESULTS

RIBA™ ANALYSIS OF DISCORDANTS



ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	3	1	2	0

Rpt. Reactive Rate

1st GEN. ELISA (c100-3) - 16%

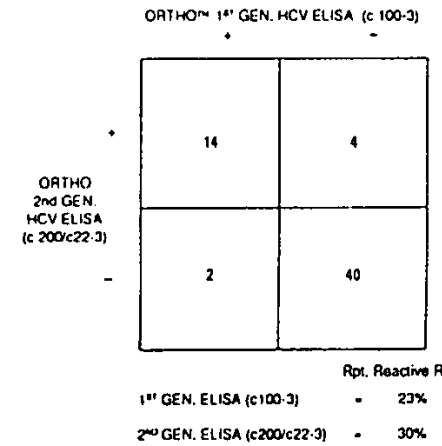
2nd GEN. ELISA (c200/c22-3) - 22%

Figure 2

MALE HOMOSEXUALS
(N = 60)

ELISA RESULTS

RIBA™ ANALYSIS OF DISCORDANTS



ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	4	4	0	0
1.0 ONLY	2	0	0	2

Rpt. Reactive Rate

1st GEN. ELISA (c100-3) - 23%

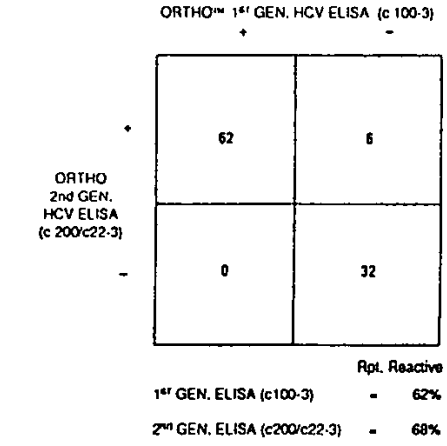
2nd GEN. ELISA (c200/c22-3) - 30%

Figure 3

ELEVATED ALT / HBc ANTIBODY NEGATIVE
(N = 100)

ELISA RESULTS

RIBA™ ANALYSIS OF DISCORDANTS



ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	6	6	0	0

Rpt. Reactive Rate

1st GEN. ELISA (c100-3) - 62%

2nd GEN. ELISA (c200/c22-3) - 68%

Figure 4

ELEVATED ALT / HBc ANTIBODY POSITIVE
(N = 58)

ELISA RESULTS

ORTHO™ 1st GEN. HCV ELISA (c 100-3)

ORTHO 2nd GEN. HCV ELISA (c 200/c22-3)	+	−
+	45	5
−	0	8

RIBA™ ANALYSIS OF DISCORDANTS

ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	5	3	2	0

Rpt. Reactive Rate

1st GEN. ELISA (c100-3)	=	78%
2nd GEN. ELISA (c200/c22-3)	=	86%

Figure 5

PAID DONORS
(N = 249)

ELISA RESULTS

ORTHO™ 1st GEN. HCV ELISA (c 100-3)

ORTHO 2nd GEN. HCV ELISA (c 200/c22-3)	+	−
+	34	8
−	2	205

RIBA™ ANALYSIS OF DISCORDANTS

ELISA REACTIVITY	No. of SPECIMENS	RIBA RESULT POS	IND	NEG
2.0 ONLY	8	1	4	3
1.0 ONLY	2	0	0	2

Rpt. Reactive Rate

1st GEN. ELISA (c100-3)	=	14%
2nd GEN. ELISA (c200/c22-3)	=	17%

Figure 6

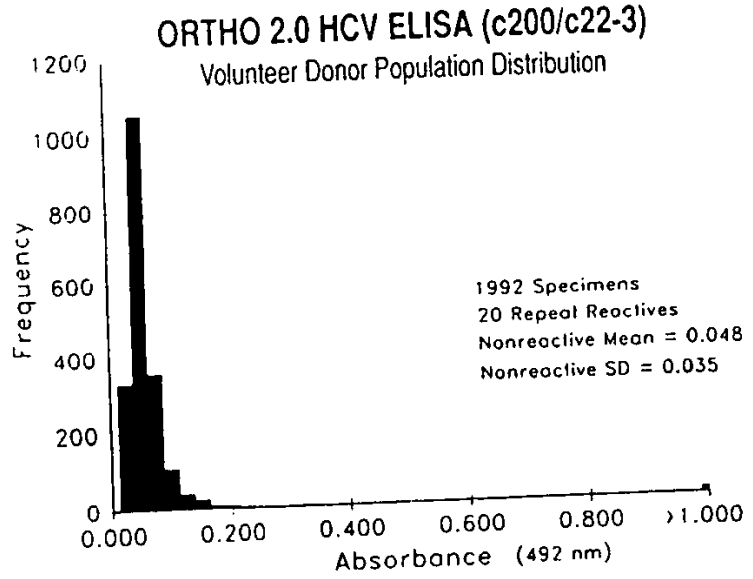


Figure 8

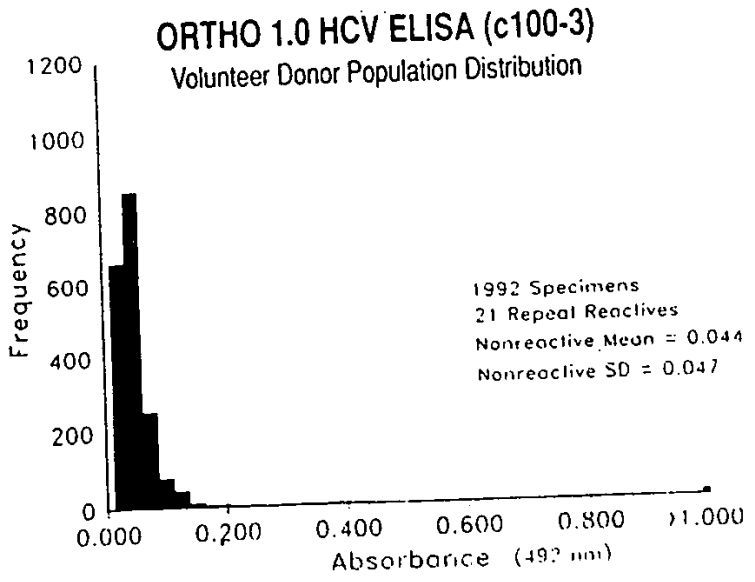


Figure 7

Presumably Healthy Volunteer donor Population

A. ELISA Results

ELISA	N	Nonreactive	Initially Reactive	Repeatably Reactive
First Gen. (c100-3)	1992	1968 (98.8%)	24 (1.20%)	21 (1.05%)
Second Gen. (c200/c22-3)	1992	1971 (98.9%)	21 (1.05%)	20 (1.00%)

B. RIBA Analysis of ELISA Repeat Reactives

ELISA Rpt Reactives	N	RIBA Results Positive	Indeterminant	Negative
First Gen. (c100-3)	21	7	3	11
Second Gen. (c200/c22-3)	20	12	5	3

Figure 9

CONCLUSIONS

1. The ORTHO second generation HCV ELISA (c200/c22-3) provides improved sensitivity for the detection of anti-HCV antibodies in individuals at high risk for HCV infection.

2. The ORTHO second generation ELISA provides an improvement in assay specificity when used to screen low risk populations for the presence of anti-HCV antibodies.

3. Use of the ORTHO second generation ELISA to screen normal volunteer blood donors would result in the detection of additional individuals with anti-HCV antibodies.