ACVSB 7/2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

AS REQUESTED

CONGRESSIONAL, INTERNATIONAL, AND CONSUMER AFFAIRS BRANCH
OFFICE OF COMPLIANCE (HFB-142)
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
PARK BUILDING - ROOM 1-58

301-443-7532

Summary of Basis for Approval

Reference Numbers: 89-0515
89-0730

Proper Name: Hepatitis C Virus Encoded Antigen

Applicant: Ortho Diagnostic Systems Inc.
Route 202
Raritan, NJ 08869

Product Tradename: ORTHO* HCV ELISA Test System

I. INDICATIONS FOR USE

ORTHO HCV ELISA Test System is a qualitative, enzyme-linked, immunosorbent assay for the detection of antibody to hepatitis C virus (anti-HCV) in human serum or plasma. The primary purpose of the assay is to screen blood donations so that units intended for transfusion containing anti-HCV can be identified and eliminated from the blood supply.

II. BRIEF DESCRIPTION OF THE TEST

ORTHO HCV ELISA Test System is an enzyme-linked immunosorbent assay (ELISA) employing a recombinant hepatitis C virus encoded antigen c100-3 (rHCV c100-3) coated onto microwell strips. During incubation with serum or plasma specimens, anti-HCV, if present, forms antigen-antibody complexes on the microwell surface. Anti-human IgG (murine monoclonal) conjugated to horseradish peroxidase is added to the microwell and binds to the

* Trademark

antigen-antibody complex. The reaction of bound conjugate with an enzyme detection system containing o-phenylenediamine and substrate results in the formation of a colored end product. The color intensity is dependent upon the amount of bound conjugate and therefore is a function of the concentration of anti-HCV present in the specimen. The color intensity is measured with a microwell spectrophotometer at 490 nm or 492 nm.

ORTHO HCV ELISA Test System consists of the following components.

1. Hepatitis C Virus (HCV) Encoded Antigen (Recombinant HCV c100-3) Coated Microwell Plates (8 strips of 12 wells each in holder)
2. PBS - phosphate-buffered saline (PBS) in crystalline form
3. Polysorbate 20 - polyoxyethylene sorbitan monolaurate
4. Specimen Diluent - phosphate-buffered saline with protein stabilizers
5. Conjugate: Antibody to Human IgG (Murine Monoclonal) - anti-human IgG (murine monoclonal) conjugated to horseradish peroxidase with protein stabilizers
6. OPD Tablets - o-phenylenediamine·2HCl
7. Substrate Buffer - 0.02% hydrogen peroxide in citrate-phosphate buffer
8. Positive Control (Human) - heat-treated serum or plasma containing anti-HCV and nonreactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1)
   Preservative: 0.02% thimerosal
9. Negative Control (Human) - serum or plasma nonreactive for HBsAg, anti-HCV and antibody to HIV-1
   Preservative: 0.02% thimerosal

III. MANUFACTURING AND CONTROLS

A. Manufacturing and Controls

ORTHO HCV ELISA Test System is prepared under U.S. License Number 156 by Ortho Diagnostic Systems Inc. under a shared manufacturing agreement with Chiron Corporation. At Chiron the RNA genome of the agent responsible for one form of non-A, non-B hepatitis (NANBH), the hepatitis C virus (HCV), was identified and the cDNA cloned. The HCV-derived recombinant polypeptide, recombinant antigen (c100-3), is the basis for the coated microwell strips for ORTHO HCV ELISA Test System. At Ortho, the HCV c100-3 antigen is diluted and coated onto microwell strips.

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 heat treated.

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 is subjected to a final performance test.

Each lot of ORTHO HCV ELISA Test System is tested with an in-house panel of samples with varying levels of anti-HCV reactivity as well as the FDA Reference Panel and must meet the performance requirements of both panels.

B. Stability Studies

The stability of ORTHO HCV ELISA Test System has been established based upon testing at the recommended storage conditions of 2 to 8°C and temperature extremes. Three lots of product were evaluated after being stored at 2 to 8°C for twelve months. These lots are continuing to be evaluated. Two lots of product were each evaluated after being subjected to temperature extremes of 37°C, 40°C and -20°C. One lot of product was shipped following standard shipping conditions and returned to Ortho for evaluation; shipping time was three-days round trip. These studies indicate no compromise in product performance to date and support a twelve-month dating period for the test kit.

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, sterility or bioburden tests and performance. Product performance is assessed through laboratory evaluations of each test kit against an in-house panel and the FDA Reference Panel containing samples that are positive for anti-HCV and samples that are negative for anti-HCV.

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 ORTHO HCV ELISA Test System is a qualitative test for detection of anti-HCV in human serum or plasma. The product tradename, ORTHO HCV ELISA Test System, is not known to conflict with any other biologic or device tradename.

E. Establishment Inspection

A prelicensing inspection of the areas where product is manufactured, tested, stored and shipped was conducted on March 27, 28 and 29, 1990. 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 treated before being used to manufacture the Positive Control.

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.

IV. BIOLOGICAL PRINCIPLES OF THE TEST

A. Principles of the Test

ORTHO HCV ELISA Test System is a three-stage test carried out in a microwell coated with a recombinant hepatitis C virus antigen.

In the first stage, a test specimen is diluted directly in the test well and incubated for a specified length of time. If anti-HCV is present in the specimen, antigen-antibody complexes will be formed on the microwell surface. If anti-HCV is not present, complexes will not be formed and the unbound serum or plasma proteins will be removed in the washing step.

In the second stage, murine monoclonal antibody conjugate is added to the microwell. The conjugate binds specifically to the anti-HCV portion of the antigen-antibody complexes. If antigen-antibody complexes are not present, the unbound conjugate will be removed by washing.

In the third stage, an enzyme detection system composed of OPD and hydrogen peroxide is added to the test well. If bound conjugate is present, the OPD will be oxidized, resulting in a colored end product. Sulfuric acid is then added to stop the reaction.

The color intensity is dependent upon the amount of bound conjugate and therefore is a function of the concentration of anti-HCV present in the specimen. The color intensity is measured with a microwell spectrophotometer at 490nm or 492nm.

B. Preclinical Data

Prior to the initiation of clinical studies, ORTHO HCV ELISA Test System was evaluated with serum and plasma specimens from presumably healthy volunteer blood donors,

specimens from high risk populations and from patients with other diseases such as rheumatoid arthritis and systemic lupus erythematosus and specimens from patients with clinically documented non-A, non-B hepatitis (NANBH) . A summary of the test results follows.

Table B.1: Presumably Healthy Volunteer Blood Donors

Population	Number Tested	Nonreactive	Initially Reactive	Repeatably Reactive
A	1000	986 (98.6%)	14 (1.4%)	12 (1.2%)
B	721	713 (98.9%)	8 (1.1%)	6 (0.8%)

Table B.2: High Risk and Other Disease Populations

Population	Number Tested	Nonreactive	Initially Reactive
Anti-HBc Reactive	200	191 (95%)	9 (5%)
Elevated ALT	100	85 (85%)	15 (15%)
Anti-HBc Reactive and Elevated ALT	50	34 (68%)	16 (32%)
IV Drug Abusers	142 (19%)	27 (81%)	115
Thalassemic	48 (54%)	26 (46%)	22
Hemophiliac	52 (37%)	19 (63%)	33
Homosexual Male	60 (80%)	48 (20%)	12
Renal Dialysis	50 (90%)	45 (10%)	5
SLE	20 (100%)	20 (0%)	0
Rheumatoid Arthritis	23 (100%)	23 (0%)	0

Specimens from clinically documented NANBH patients were tested as a coded panel.

The panel included proven infectious sera from chronic human NANBH carriers,

infectious sera from implicated donors and infectious sera from acute phase NANBH patients.

The results are summarized as follows.

Specimen Description	Number Tested	Nonreactive	Reactive
1. Proven Infectious by Chimpanzee Transmission			
Chronic NANBH, Post Transfusion	3	0	3
Implicated Donors with Elevated ALT	3	0	3
Acute NANBH, Post-Transfusion	1	1	0
2. Equivocally Infectious by Chimpanzee Transmission			
Implicated Donor with Normal ALT	1	1	0
3. Acute NANBH, Post-Transfusion	3	2	1

V. CLINICAL DATA

A. Summary of Clinical Data

Ten independent clinical study sites tested a total of 34,038 specimens from blood donors, source plasma donors, high risk individuals, non-A, non-B hepatitis (NANBH) patients, NANBH controls and others. Of these, 9998 specimens were from blood donors and 10,523 specimens were from source plasma donors. The clinical study sites included large volunteer blood banks, commercial plasmapheresis locations and clinical and research centers. The results of reactivity with ORTHO HCV ELISA Test System are summarized as follows.

Reactivity in Volunteer Blood Donors

The ability of ORTHO HCV ELISA Test System to detect anti-HCV among presumably healthy volunteer blood donors is shown in Table 1. The data represent 3999 fresh serum specimens (sites 1 and 2) and 5999 fresh plasma specimens (sites 3 and 4) from four geographically distinct locations within the United States (California, N. Carolina, Arizona, Missouri).

Table 1: Detection of Anti-HCV in Serum and Plasma from Volunteer Blood Donors

Site	Number Tested	Nonreactive	Initially Reactive	Repeatably Reactive
1	2000	1984 (99.2%)	16 (0.8%)	12 (0.6%)
2	1999	1977 (98.9%)	22 (1.1%)	20 (1.0%)
3	1999	1989 (99.5%)	10 (0.5%)	8 (0.4%)
4	4000	3981 (99.5%)	19 (0.5%)	15 (0.4%)
TOTAL	9998	9931 (99.3%)	67 (0.7%)	55 (0.6%)

ALT and anti-HBc results for the 55 anti-HCV repeatably reactive donors identified in screening 9998 blood donors are shown in Table 2. Thirty-nine (39) of the 55 donors (70.9%) were only anti-HCV reactive, that is, they had normal ALT levels and were nonreactive for anti-HBc.

Table 2: ALT and Anti-HBc Results for Anti-HCV Repeatably Reactive Donors

Marker	Number Tested	Repeatably Reactive
Elevated ALT only	7	12.7%
Anti-HBc only	4	7.3%
Elevated ALT and Anti-HBc	5	9.1%
Anti-HCV only	39	70.9%
TOTAL	55	100.0%

Reactivity in Commercial Plasma Donors

The ability of ORTHO HCV ELISA Test System to detect anti-HCV among presumably healthy commercial plasma donors is shown in Table 3. The data represent 10,523 fresh plasma specimens from nineteen geographically distinct locations within the United States. The specimens were tested at four clinical sites.

Table 3: Detection of Anti-HCV in Commercial Plasma Donors

Site	Number Tested	Nonreactive	Initially Reactive	Repeatably Reactive
1	2520	2352 (93.3%)	168 (6.7%)	165 (6.5%)
2	3000	2821 (94.0%)	179 (6.0%)	171 (5.7%)
3	3000	2810 (93.7%)	190 (6.3%)	182 (6.1%)
4	2003	1815 (90.6%)	188 (9.4%)	185 (9.2%)
TOTAL	10523	9798 (93.1%)	725 (6.9%)	703 (6.7%)

Reactivity in High Risk Populations

Anti-HCV reactivity in populations at risk for acquiring/transmitting NANBH was studied at four clinical sites. Results appear in Table 4.

Table 4: Detection of Anti-HCV in High Risk Populations

Risk Group	Number Tested	Repeatably Reactive
IV Drug Abusers	414	313 (75.6%)
Renal Dialysis Patients	697	141 (20.2%)
Hemophilia Patients	184	110 (59.8%)
Thalassemia Patients	40	6 (15.0%)
Homosexual Males	388	17 (4.4%)

Reactivity in Transfusion Recipients with TA-NANBH

The ability of ORTHO HCV ELISA Test System to detect anti-HCV in prospectively followed transfusion recipients with clinically diagnosed transfusion associated-NANBH (TA-NANBH) is shown in Table 5. These samples were collected as part of the Transfusion-Transmitted Viruses Study[1] and the NIH NANBH Study[2,3].

The recipients were categorized as acute, indeterminate or chronic ALT elevations based on bleed date and ALT levels only.

Acute: Pre-transfusion ALT level within the normal range, followed by a post-transfusion elevated ALT level which returned to normal and remained normal during follow-up

Chronic: ALT levels which remained elevated following transfusion for at least six months

Indeterminate: ALT results which did not meet the criteria for either acute or chronic

The results for the control group, prospectively followed transfusion recipients who did not develop clinically diagnosed TA-NANBH, are also shown in Table 5.

Table 5: Detection of Anti-HCV in Transfusion Recipients

Category	Number of Recipients	Repeatably Reactive
Acute TA-NANBH	57	18 (31.6%)
Indeterminate TA-NANBH	21	15 (71.4%)
Chronic TA-NANBH	63	51 (81.0%)
Controls	122	4 (3.3%)

For the recipients listed in Table 5 specimens were taken pre-transfusion and at 3, 6 and 9 to 12 months post-transfusion.

There were complete donor sets (that is, all units received by recipient were available for testing) for 242 of the 263 TA-NANBH and control recipients listed in Table 5. Only partial donor sets were available for the remaining 21 recipients.

Seventy-six of the 242 recipients (65 clinically diagnosed TA-NANBH [65/120 NANBH patients, 54%] and 11 controls who did not develop TA-NANBH [11/122 controls, 9%]) received transfusions from at least one donor who was anti-HCV reactive. Of the 65 (85.5%) clinically diagnosed TA-NANBH, 56 (86.1%) were also anti-HCV reactive.

Reactivity in well-pedigreed Patients with a Diagnosis of NANBH

Sixty-one patients were enrolled in a study conducted at the National Institutes of Health to determine the efficacy of a-interferon in the treatment of NANB hepatitis. Eighteen (18) of the patients tested were taken from the Phase 1 open trial (all treated with a-interferon), with the remaining 43 participants being drawn from the randomized Phase 2 placebo-controlled trial.

Fifty-nine patients with pre and post-treatment specimens were included in this study. The remaining two patients withdrew from the study and thus had only one pretreatment sample available. These two patients have been included in the placebo group for the sake of this analysis. Liver biopsy results were available on all study participants. All samples tested were frozen sera, collected between July 1984 and February 1989. The average time between bleedings on a single patient was six months.

The patients could be categorized based on liver biopsy results which included Chronic Persistent Hepatitis (CPH), Chronic Active Hepatitis (CAH), and Cirrhosis (CIR), and according to the drug given (Placebo or a-Interferon) as presented in Table 6.

Table 6: NANBH/a-Interferon Study Patients at Study Entry (anti-HCV reactive/total patients tested)

N=61

	CPH	CAH	CIR	Total (%)
Placebo	2/2	15/17	3/3	20/22 (90)
a-Interferon	4/5	21/23	9/11	34/39 (87)
Total	6/7	36/40	12/14	54/61 (88)

The overall rate of anti-HCV reactivity in this study was 88.5%, with a 95% confidence interval ranging from 76.4% to 95.4%. All but three of the patients with a reactive anti-HCV result pretreatment remained reactive in the later post-treatment sample. The three patients with reactive pre-treatment and nonreactive post-treatment samples are as follows:

1. CAH/Interferon, with samples taken 6 months apart
2. CAH/Interferon, with samples taken 13 months apart
3. CIR/Interferon, with samples taken 2 months apart

B. Reproducibility

A reproducibility panel comprised of three members with different anti-HCV reactivity (nonreactive, weakly reactive and strongly reactive) was tested at four sites on each of three days. Intra-assay results are summarized in the following table. The intra-assay SD is the standard deviation between absorbance values within strip, plate and lot.

Ortho Diagnostic Systems Inc
Hepatitis C Virus Encoded Antigen
May 1990

Reproducibility Panel Results

Site	Parameter	Nonreactive	Weakly Reactive	Strongly Reactive
1	N	168	168	168
	Mean Absorbance	0.009	0.659	1.407
	SD	0.017	0.100	0.165
	% CV	ND[1]	15	12
2	N	252	252	252
	Mean Absorbance	0.030	0.951	1.726
	SD	0.008	0.132	0.144
	% CV	ND	14	8
3	N	252	252	252
	Mean Absorbance	0.024	0.817	1.355
	SD	0.011	0.127	0.155
	% CV	ND	16	11
4	N	252	252	252
	Mean Absorbance	.031	1.034	1.577
	SD	0.007	0.107	0.115
	% CV	ND	10	7

[1]ND = Not determined (CV for nonreactive specimen is not meaningful since the mean absorbance value lies close to zero.)

C. Performance Characteristics

Consistency in the production of test kits has been demonstrated by testing at least three production lots with the FDA Reference Panel.

VI. PACKAGE INSERT

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

VII. REFERENCES

1. Aach RD, Szmuness W, Mosley JW, et al. Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients: the Transfusion-Transmitted Viruses Study. New England Journal of Medicine 1981; 304:989.
2. Alter HJ, Purcell RH, Holland PV, et al. Transmissible Agent in Non-A, Non B Hepatitis. Lancet. 1978: 459-463.
3. Koziol DE, Holland PV, Alter HJ, et al. Antibody to Hepatitis B Core Antigen as a Paradoxical Marker for Non-A, Non-B, Hepatitis Agents in Donated Blood. Annals of Internal Medicine, 104(4); April 1986.