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Hepatitis C Virus Encoded Antigen (Recombinant c22-3, c200 and NS5)
ORTHO® HCV Version 3.0 ELISA Test System

Enzyme-Linked Immunosorbent Assay for the Detection of Antibody to Hepatitis C Virus (Anti-HCV) in Human Serum or Plasma

REF

480 Test Kit: 930740
2400 Test Kit: 930750

Rx ONLY

INTENDED USE

ORTHO® HCV Version 3.0 ELISA Test System is an enzyme-linked, immunosorbent assay for the qualitative detection of antibody to hepatitis C virus (anti-HCV) in human serum, plasma, and cadaveric specimens.

This product is intended for use as a donor screening test to detect antibodies to hepatitis C virus in plasma and serum samples from individual human donors, including volunteer donors of Whole Blood, blood components, source plasma, and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. This test is not intended for use on samples of cord blood.

The ORTHO® HCV Version 3.0 ELISA Test System is intended for use in a fully manual mode, in semi-automated mode using the ORTHO VERSEIA® Pipetter, or in automated mode with the ORTHO® Summit System (OSS).

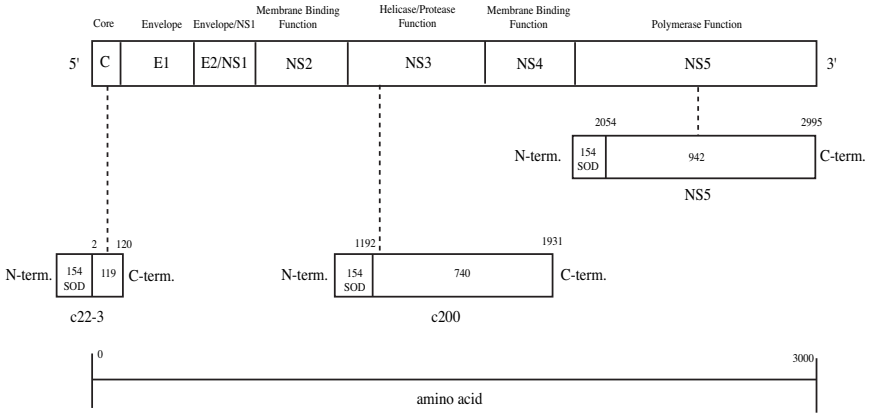
SUMMARY AND EXPLANATION

ORTHO® HCV Version 3.0 ELISA Test System is an enzyme-linked immunosorbent assay (ELISA) which utilizes microwells coated with recombinant hepatitis C virus encoded antigens as the solid phase. ELISA technology utilizes the principle that antigens or antibodies which become bound to the solid phase can be detected by complementary antibody or antigen which is labeled with an enzyme capable of acting on a chromogenic substrate. When enzyme substrate is applied, the presence of antigen or antibody can be detected by the development of a colored end product. Immunoassays of this type were first developed in the early 1970s.[1] Since that time, ELISA technology has been extensively used for the detection of antigens and antibodies for a wide range of infectious diseases.

Three recombinant hepatitis C virus encoded antigens are used in ORTHO® HCV Version 3.0 ELISA Test System. The three recombinant antigens, developed by Novartis Vaccines and Diagnostics, Inc., are c22-3, c200 and NS5. A graphic representation of the putative HCV genome and recombinant proteins appears in Figure 1.

Figure 1

HCV Genome and Recombinant Proteins



HCV recombinant protein c22-3 is encoded by the putative core region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c22-3 is derived from a structural region of the genome which encodes the RNA-binding nucleocapsid protein. Nucleocapsid proteins are thought to be involved in forming the viral core structure. Recent studies have indicated that antibodies which develop following infection with HCV are often reactive with c22-3.[3] Moreover, studies performed using the CHIRON® RIBA® HCV 2.0 Strip Immunoblot Assay (SIA) for anti-HCV have shown that in many cases antibodies to c22-3 develop sooner following HCV infection than those to c100-3.[4]

HCV recombinant protein c200 is encoded by the putative NS3 and NS4 regions of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c200 is derived from nonstructural regions of the genome. The c200 recombinant protein contains the c33c protein sequence genetically linked to the c100-3 protein sequence.

c33c is encoded by the putative NS3 portion of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that the NS3 region encodes the viral helicase, an enzyme involved in the unwinding of RNA during replication of the viral genome by RNA-dependent RNA polymerase. Recent studies have indicated that antibodies which develop following infection with HCV are frequently reactive with c33c. Studies performed using the CHIRON® RIBA® HCV 2.0 SIA for anti-HCV have shown that antibodies reactive with c33c often develop sooner following HCV infection than do those to c100-3.[4]

HCV recombinant protein c100-3 is encoded by the putative NS4 region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c100-3 is derived from a nonstructural region of the genome. At present, the function of this portion of the HCV genome is unknown. Antibodies which develop following infection with HCV are often reactive with c100-3.[2]

HCV recombinant protein NS5 is encoded by the putative NS5 region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that NS5 is derived from a nonstructural region of the genome that encodes the viral polymerase, an enzyme involved in replication of HCV. Recent studies have indicated that a significant proportion of persons infected with HCV develop antibodies to NS5.[5,6]

The use of HCV recombinant proteins derived from the core, NS3, NS4 and NS5 regions of the HCV genome has shown to be effective in identifying a greater number of diagnosed acute and chronic non-A, non-B hepatitis patients than single antigen (c100-3) assays.[5,6] In addition, the use of these additional proteins allows for earlier detection of seroconversion following HCV infection. Although antibody responses to NS5 region-encoded antigens are not as prevalent in response to HCV infection as those to core and NS3 region-encoded antigens, the addition of NS5 to c22-3 and c200 recombinant proteins in ORTHO® HCV Version 3.0 ELISA Test System affords antibody detection to a greater number of HCV-encoded epitopes.

The amino acid sequence of the three HCV recombinant proteins is as follows.

Recombinant Protein	Polyprotein Sequence
c22-3	AA # 2-120
c200	AA # 1192-1931
NS5	AA # 2054-2995

The host organism for all three HCV recombinant proteins is *S. cerevisiae* (yeast).

The primary purpose of this assay is to screen blood donations so that units containing HCV antibody can be identified and eliminated from the blood supply. Although the presence of anti-HCV does not constitute a diagnosis of HCV infection, the determination of anti-HCV may be used as an aid in the diagnosis of hepatitis C and in the differential diagnosis of non-A, non-B hepatitis in conjunction with determination of liver enzymes, additional serological markers and clinical evaluation. The Hepatitis C Virus Encoded Antigen (Recombinant c22-3, c200 and NS5) used in the manufacture of ORTHO® HCV Version 3.0 ELISA Test System is prepared under U.S. License by Novartis Vaccines and Diagnostics, Inc. under a shared manufacturing arrangement.

PRINCIPLE OF THE PROCEDURE

The assay procedure is a three-stage test carried out in a microwell coated with a combination of recombinant hepatitis C virus (rHCV) antigen (c22-3, c200 and NS5).

In the first stage, a diluted test specimen is incubated in the test well for a specified length of time. If antibody reactive to any of the three antigens 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. In the subsequent washing step, unbound serum or plasma proteins will be removed.

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

In the third stage, an enzyme detection system composed of *o*-phenylenediamine (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. In this reaction, peroxidase is divalently oxidized by hydrogen peroxide to form an intermediate compound, which is, in turn, reduced to its initial state by subsequent interaction with hydrogen ion donating OPD. The resulting oxidized form of OPD has an orange color. 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 reader (photometer) designed to measure light absorbance in a microwell.

REAGENTS

Label Abbreviations	480 Test Kit Product Code 930740	2400 Test Kit Product Code 930750	Component Description
HCV	5 plates	25 plates	Hepatitis C Virus (HCV) Encoded Antigen (Recombinant c22-3, c200 and NS5) – Coated Microwell Plates (96 wells each) – c22-3, c200 and NS5 derived from yeast
CON	1 bottle (125 mL)	5 bottles (125 mL each)	Conjugate – Antibody to Human IgG (Murine Monoclonal) – anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers Preservative: 1% ProClin® 300
SD	1 bottle (190 mL)	4 bottles (190 mL each)	Specimen Diluent – phosphate-buffered saline with bovine protein stabilizers Preservative: 0.1% 2-chloroacetamide
PC	1 vial (1.0 mL)	4 vials (1.0 mL each)	Positive Control (Human) Source: Treated human serum or plasma containing anti-HCV and nonreactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). The anti-HCV serum or plasma has been treated to reduce the titer of potentially infectious virus. However, no test method can rule out the risk of potential infection; **handle as if capable of transmitting infection.** Preservative: 0.2% sodium azide
NC	1 vial (1.5 mL)	5 vials (1.5 mL each)	Negative Control (Human) Source: Human serum or plasma nonreactive for HBsAg, antibody to HIV-1, antibody to HIV-2 and anti-HCV Preservative: 0.2% sodium azide
OPD	1 vial (30 tablets)	5 vials (30 tablets each)	OPD Tablets – contains *o*-phenylenediamine • 2HCl
SB	1 bottle (190 mL)	4 bottles (190 mL each)	Substrate Buffer-G – citrate-phosphate buffer with 0.02% hydrogen peroxide Preservative: 0.1% 2-chloroacetamide
	21	84	Plate Sealers, disposable

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

STORAGE REQUIREMENT

Store unopened and opened components at 2 to 8°C

FOR IN VITRO DIAGNOSTIC USE

ORTHO® HCV Version 3.0 ELISA Test System meets the FDA potency requirements.

PRECAUTIONS

1. **CAUTION:** Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be handled as potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory practices.[7,8]
2. Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterward.
3. All specimens should be handled as potentially infectious agents.
4. Sodium azide is included as a preservative in the Positive Control and Negative Control. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. These azides are potentially explosive. To prevent buildup, flush plumbing with a large volume of water while disposing of these solutions in the sink.
5. Handle and dispose of all specimens and materials used to perform the test as if they contain infectious agents. Disposal of all specimens and materials should comply with all local, state and federal waste disposal requirements.[10,11]
6. 4N sulfuric acid (H_2SO_4) (CAS 7664-93-9) is a strong acid. Wipe up spills immediately. Flush the area of the spill with water. If the acid contacts the skin or eyes, flush with copious amounts of water and seek medical attention. Following are the Hazard and Precautionary Requirements.[9] Refer to www.orthoclinical.com for the Safety Data Sheets and for OCD contact information.

DANGER:

Hazard Statements:
Toxic if inhaled.
Causes severe skin burns and eye damage.

Precautionary Statements:
Use only outdoors or in a well-ventilated area.
Do not breathe dust/fume/gas/mist/vapors/spray.
Wash face, hands and any exposed skin thoroughly after handling.
Wear protective gloves/protective clothing/eye protection/face protection.
Immediately call a POISON CENTER or doctor/physician.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Immediately call a POISON CENTER or doctor/physician.
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or doctor/physician.
IF SWALLOWED: Rinse mouth. DO NOT induce vomiting.

7. Handle OPD tablets with plastic or Teflon®-coated forceps only. Metal forceps may react with tablets and interfere with the test results. The vial cap may be used for counting and adding tablets.
8. Avoid contact of OPD with eyes, skin or clothing, as OPD may cause irritation or an allergic skin reaction. If OPD should come into contact with the skin, wash thoroughly with water. OPD is toxic for inhalation, ingestion, and skin contact. In case of malaise, call a physician.
9. OPD tablets are light - and moisture-sensitive. Keep vial **tightly** closed when not in use. **Bring vial to room temperature (15 to 30°C) before opening.** The desiccant pouch must be retained in the vial at all times. Do not use tablets which are yellow or broken.
10. *o*-Phenylenediamine (CAS 95-54-5) dihydrochloride is included in the OPD tablet. Following are the Hazard and Precautionary Requirements.[9] Refer to www.orthoclinical.com for the Safety Data Sheets and for OCD contact information.

DANGER:

Hazard Statements:
Toxic if swallowed.
Harmful in contact with skin.
Harmful if inhaled.
Causes serious eye irritation.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
Suspected of causing cancer.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Use only outdoors or in a well-ventilated area.
Wash face, hands and any exposed skin thoroughly after handling.
Do not eat, drink or smoke when using this product.
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Use personal protective equipment as required.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves/protective clothing/eye protection/face protection.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
IF ON SKIN: Wash with plenty of soap and water. Call a POISON CENTER or doctor/physician if you feel unwell. Wash contaminated clothing before reuse. If skin irritation or rash occurs: Get medical advice/attention.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.

11. ProClin® 300 (CAS 55965-84-9) is included as a preservative in the Conjugate. Following are the Hazard and Precautionary Requirements.[9] Refer to www.orthoclinical.com for the Safety Data Sheets and for OCD contact information.

WARNING:

Hazard Statement:
May cause an allergic skin reaction.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves.
IF ON SKIN: Wash with plenty of soap and water.
If skin irritation or rash occurs: Get medical advice/attention.
Wash contaminated clothing before reuse.

12. 2-Chloroacetamide (CAS 79-07-2) is included as a preservative in the Specimen Diluent, 20X Wash Buffer Concentrate and Substrate Buffer-G. Following are the Hazard and Precautionary Requirements.[9] Refer to www.orthoclinical.com for the Safety Data Sheets and for OCD contact information.

WARNING:

Hazard Statements:
May cause an allergic skin reaction.
Suspected of damaging fertility or the unborn child.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves.
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Use personal protective equipment as required.
IF ON SKIN: Wash with plenty of soap and water.
If skin irritation or rash occurs: Get medical advice/attention.
Wash contaminated clothing before reuse.

13. Distilled or deionized water must be used for Wash Buffer preparation. Clinical laboratory reagent water Type I or Type II is acceptable.[12] Store the water in nonmetallic containers.
14. Do not mix lot numbers of coated microwell plates, Specimen Diluent, Conjugate Reagent, Negative Control, or Positive Control from kits with different lot numbers. Any lot number of Substrate Buffer-G, OPD tablets, 4N sulfuric acid (H_2SO_4), and 20X Wash Buffer Concentrate may be used provided they are not used beyond the labeled expiration date.
15. All reagents and components **must** be at room temperature prior to use and kit components returned to 2 to 8°C after use.
16. The microwell strips are sealed in protective pouches with a humidity indicator desiccant. The desiccant, normally blue/purple in color, will turn pink if moisture is present in the pouch. If the desiccant is pink, the microwell strips should not be used.
17. Desiccant is included in both OPD tablet and Microwell Plate. Synthetic amorphous precipitated silica gel (SiO2) (CAS 112926-00-8) and Cobalt chloride (CAS 7646-79-9) are included in the desiccant. Following are the Hazard and Precautionary Requirements.[9] Refer to www.orthoclinical.com for the Safety Data Sheets and for OCD contact information.

    DANGER:

    Hazard Statements:
    May cause cancer.
    May damage fertility.
    Toxic to aquatic life.
    Harmful to aquatic life with long lasting effects.

    Precautionary Statements:
    Obtain special instructions before use.
    Do not handle until all safety precautions have been read and understood.
    Avoid release to the environment.
    Wear protective gloves.
    Use personal protective equipment as required.
    If exposed or concerned: Get medical advice/attention.
    Store locked up.
    Dispose of contents/container in accordance with local/regional/national/international regulations.
18. Do not use reagents beyond their labeled expiration date.
19. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
20. Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result.
21. When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multichannel micropipette, new tips are to be used for each reagent to be added.
22. Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance (see Step 7 of Test Procedure).
23. Do not allow microwells to become dry once the assay has begun.
24. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells. If necessary, wipe the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading.
25. Ensure that the microwell strips are level in the microwell strip holder during the test procedure.
26. Negative or positive control values which are not within the expected range (refer to **Quality Control Procedures** section) may indicate a technique problem or product deterioration.
27. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell strips during the assay as the color reaction may be inhibited.
28. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
29. The microwell reader should contain a reference filter with a setting at 620 nm or 630 nm. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched or irregular may cause elevated readings.
30. Delays in plate processing may affect absorbance values.
31. Room temperature is defined as 15 to 30°C.
32. Serum-separator tubes (SST) or plasma preparation tubes (PPT) should be used with caution when using automated pipetting instrumentation. Consult the Instrument User's Manuals for precautions.
33. Refer to "Precautions" in other ORTHO® instruments User's Manuals:
    a. ORTHO® Summit System User's Guide
    b. ORTHO VERSEIA® Pipetter User's Guide
    c. ORTHO® Summit Processor User's Guide
    d. AutoReader IV User's Guide
    e. Model 120 Incubator Operator's Manual
    f. ORTHO® Training and Reference Manual
34. Visual inspections of the reagents should be performed prior to use to check for color change, cloudiness, and precipitates.

PREPARATION OF REAGENTS

1. **Preparation of Wash Buffer (1X):** Mix 1 part of 20X Wash Buffer Concentrate with 19 parts of distilled or deionized water (1 to 20 dilution). Wash Buffer (1X) is stable for 30 days when stored at room temperature. For longer storage (up to 60 days), keep at 2 to 8°C. Record the date the Wash Buffer (1X) is prepared and the expiration date on the container. Discard Wash Buffer (1X) if visibly contaminated.

   NOTE: Any lot number of 20X Wash Buffer Concentrate may be used to prepare this reagent provided it is not used beyond its labeled expiration date.

2. **Preparation of Substrate Solution:** Clean glass or plastic vessels must be used. Prior to the end of the second incubation, transfer a **sufficient** amount of Substrate Buffer-G to a container and protect the contents from light. **Completely dissolve** the appropriate number of OPD tablets in Substrate Buffer-G prior to use. Each microwell plate requires at least 20 mL of Substrate Solution. More Substrate Solution may be needed depending upon the reagent dispenser used. See the instrument manufacturer's instructions for additional reagent requirements. Below are guidelines for general use.

Number of Wells	Number of Plates	Number of OPD Tablets	Substrate Buffer-G (mL)
24	0.25	1	6
48	0.5	2	12
72	0.75	3	18
96	1	4	24
192	2	7	42
288	3	10	60

The Substrate Solution is stable for 8 hours after the addition of OPD tablets when held at room temperature **in the dark**. Record the time when the OPD tablets are added to the Substrate Buffer-G and when it will expire on the container. **Do not use more than a single preparation of Substrate Solution per plate.**

SPECIMEN COLLECTION, STORAGE, AND HANDLING

NOTE: Handle all specimens as if they are capable of transmitting infectious agents.

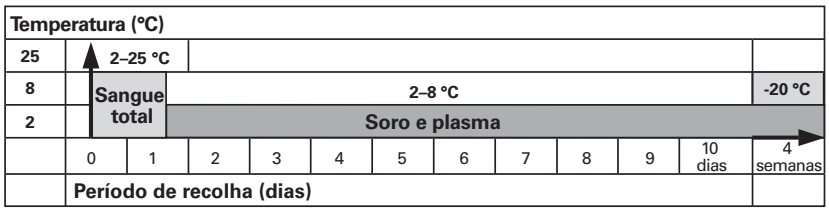
Living Donor Specimens

A. Blood specimens collected in glass, plastic, or serum-separator tubes may be used.

B. Plasma specimens collected in EDTA (glass and plastic tubes, plasma preparation tubes), lithium heparin, CPD, CP2D, CPDA-1, ACD, or 4% citrate anticoagulants may be used. Plasma collected with an improper ratio of specimen to anticoagulant should not be used.

C. Whole blood may be stored up to 25°C for 24 hours from time of draw and serum and EDTA plasma specimens may be stored up to 10 days from time of draw at 2 to 8°C prior to centrifugation. Do not freeze whole blood.

D. Specimens may be stored for up to 10 days from time of draw at 2 to 8°C following centrifugation, or up to 4 weeks at -20°C undergoing 5 freeze/thaw cycles. Store specimens in appropriately qualified freezers. Mix specimen thoroughly after thawing and before testing.



E. Studies have demonstrated that specimens may be shipped at ambient temperature (up to 37°C) for up to seven days or refrigerated (2 to 8°C) for up to seven days. Upon arrival, specimens should be stored at 2 to 8°C. For shipments requiring extensive transit times (greater than seven days), specimens should be kept frozen (-20°C or below).

F. If specimens are to be shipped, they must be packaged in compliance with International Air Transport Association (IATA) and other applicable guidelines and regulations.[13,16]

G. No special preparation of the donor is required prior to specimen collection. Blood should be collected by approved medical techniques. Proper sample handling techniques should be employed to avoid microbial contamination.

H. Clear, non-hemolyzed samples are preferred. Precipitates in specimens should be removed by centrifugation.

I. When pipetting samples in an automated mode, presence of any clot, particulate matter or bubble may give an invalid test with no test results generated. All specimens should be cleared of any clot, particulate matter or bubbles before pipetting.

J. No effect on reactivity was observed when 30 HCV reactive and 30 nonreactive specimens were treated with up to 800 mg/dL of hemoglobin and 30 mg/dL of bilirubin.

K. No effect on reactivity was observed for lipids when 30 HCV reactive and 30 nonreactive specimens were treated with up to 3000 mg/dL of triglyceride.

L. No effect on reactivity was observed in 20 nonreactive specimens containing ≥9.0 g/dL total protein.

M. No interference from human anti-mouse antibodies (HAMA) was observed in a 15 member commercially available HAMA panel. No interference from heterophilic antibodies was observed in a 15 member commercially available panel.

N. **Do not use heat-treated specimens.**

O. Specimens such as pleural fluids, saliva, urine, and nonhuman specimens have not been evaluated with this assay and should not be used.

Cadaveric Donor Specimens

P. Cadaveric specimens may be collected into serum, serum-separator tubes, or EDTA blood collection devices.

Q. Cadaveric specimens may be stored for up to 10 days at 2 to 8°C and up to 4 weeks at -20°C undergoing 5 freeze/thaw cycles. Store specimens in appropriately qualified freezers. Specimens may be frozen and thawed up to 5 times. Mix specimen thoroughly after thawing and before testing.

R. Studies have demonstrated that specimens may be shipped at ambient temperature (up to 37°C) for up to seven days or refrigerated (2 to 8°C) for up to seven days. Upon arrival, specimens should be stored at 2 to 8°C. For shipments requiring extensive transit times (greater than seven days), specimens should be kept frozen (-20°C or below).

S. If specimens are to be shipped, they must be packaged in compliance with International Air Transport Association (IATA) and other applicable guidelines and regulations.[13,16]

T. Proper sample handling techniques should be employed to avoid microbial contamination.

U. Clear, non-hemolyzed samples are preferred. Precipitates in specimens should be removed by centrifugation.

V. No effect on reactivity was observed when the level of hemolysis in the cadaveric specimens ranged from 0 mg/dL to 800 mg/dL of hemoglobin.

Specimen Pooling

Testing of these specimens is not recommended. No data are available to interpret tests performed on pooled blood or processed plasma and products made from such pools.

PROCEDURE

Operational Modes

Manual testing is performed with handheld pipette sample handling, AutoReader IV, Model 120 Incubator or equivalent microwell incubator capable of maintaining 37°C, and ORTHO® Assay Software (OAS).

Automated testing is performed with the ORTHO® Summit System (OSS), defined as the ORTHO VERSEIA® Pipetter, ORTHO® Summit Processor (OSP), and ORTHO® Assay Software (OAS).

Semi-automated testing is performed with the ORTHO VERSEIA® Pipetter, AutoReader IV, Model 120 Incubator or equivalent microwell incubator capable of maintaining 37°C, and ORTHO® Assay Software (OAS).

Under circumstances of limited sample volume or limited number of samples, handheld pipette sample handling may be combined with the ORTHO® Summit Processor (OSP) and ORTHO® Assay Software (OAS).

An ORTHO® Assay Protocol Disk (OAPD) for ORTHO® HCV Version 3.0 ELISA Test System is also used in the testing of the samples by all processing methods.

The protocol to run this test on the ORTHO® Summit System (OSS) is contained on the ORTHO® HCV Version 3.0 ELISA Test System ORTHO® Assay Protocol Disk (OAPD) for the ORTHO® Assay Software (OAS). The pipetting protocol for the ORTHO VERSEIA® Pipetter is provided by the ORTHO VERSEIA® Pipetter software.

Follow the instructions in the OSS User's Guide.

Materials Provided

480 Test Kit (Product Code 930740)
2400 Test Kit (Product Code 930750)

Materials Required But Not Provided

- ORTHO® Assay Protocol Disk (OAPD) for ORTHO® HCV Version 3.0 ELISA Test System (Product Code 938220)
- ORTHO® HCV Version 3.0 ELISA Test System Plate Bar Code Labels (Product Code 6902520, 1000 pkg and 6902521, 4500 pkg) required to perform the assay on the ORTHO® Summit System
- ORTHO® HCV Version 3.0 ELISA Test System Control Vial Bar Code Label Sets (Product Code 6902523, 150 Sets of Control Vial Labels) required to perform the assay on the ORTHO VERSEIA® Pipetter
- ORTHO® HCV Version 3.0 ELISA Test System Specimen Diluent Reagent Container Bar Code Label Sets (Product Code 6902524, 150 Sets of Specimen Diluent Labels) required to perform the assay on the ORTHO VERSEIA® Pipetter
- ORTHO® Summit System User's Guide (Product Code 936578) and other appropriate OSS user documentation listed in the guide to run the assay on OSS
- ORTHO® Summit Processor, adjustable multichannel micropipettes, or equivalent reagent dispenser capable of delivering 50 µL and 200 µL with at least ± 5% accuracy
- ORTHO VERSEIA® Pipetter, a micropipette, or equivalent pipetter-dilutor capable of delivering 10 µL to 15µL with at least ± 10% and 200 µL to 300 µL with at least ± 5% accuracy
- 10 µL to 300 µL disposable pipette tips or equivalent
- Appropriately sized serological pipette or graduated cylinder
- Multichannel micropipette reservoirs or equivalent reagent containers
- ORTHO® Summit Processor or a multichannel microwell aspirator-washer device capable of at least 5 cycles of wash by dispensing and aspirating 300 µL to 800 µL of fluid per well and leaving a full well of fluid to soak at least 20 seconds. (Consult the device operator's manual for additional technical information.)
- ORTHO® Summit Processor or AutoReader IV or a dual wavelength microwell reader capable of reading at 490 or 492 nm with a reference filter of 620 or 630 nm. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched, or irregular may cause erroneous readings. Linearity of the microwell reader must range from at least 0 to 2.5 absorbance units. Consult the instrument manufacturer's specifications.
- ORTHO® Summit Processor or equivalent 37°C ± 1°C microwell incubator (dry or humidified)
- 20X Wash Buffer Concentrate (Product Code 933730) - phosphate buffer with sodium chloride and detergent.

  Preservative: 2% 2-chloroacetamide.
- 4N Sulfuric Acid (H_2SO_4) - available in the United States from Ortho-Clinical Diagnostics, Inc. (Product Code 933040) or equivalent.

  NOTE: To determine the suitability of another source of acid, prepare Substrate Solution as described under **PREPARATION OF REAGENTS**. Add 200 µL of Substrate Solution to three microwells, and then add 50 µL of 4N Sulfuric Acid (H_2SO_4) to be tested to each microwell. Read the microwells at a wavelength of 490 or 492 nm with a reference filter of 620 or 630 nm at "0" time and "60 minutes." All absorbance values at each time interval must be less than or equal to 0.050.
- Distilled or deionized water; clinical laboratory reagent water Type I or Type II is acceptable. (See the **PRECAUTIONS** section.)
- 5.25% sodium hypochlorite (chlorine bleach)
- Plastic or Teflon®-coated forceps
- White microwell strips (Product Code 50000312, Ortho-Clinical Diagnostics, Inc.) or equivalent uncoated microwells

Test Procedure

1. Approximately **30 minutes** prior to the beginning of the procedure, bring kit components to room temperature (15 to 30°C). Invert liquid reagents gently several times, but avoid foaming. Check the incubator temperature; maintain at 37°C ± 1°C.

2. Determine the total number of wells needed for the assay. In addition to specimens, one substrate blank, three negative controls and two positive controls must be included on each plate or partial plate. Unused wells should be stored at 2 to 8°C in the supplied foil pouch **with desiccant**, tightly sealed and used within 42 days of opening the foil pouch. Record the date the pouch is opened and the expiration date of the unused wells in the space provided on the pouch.

   Performing the test on less than a full plate is permitted as long as the following conditions are met.

   Microwell strips from different plates can be mixed to assemble full or partial plates as long as they are from the same lot, within the open pouch expiration date and have come from plates that have previously demonstrated proper response to kit controls.

   When assembling a plate which contains strips from a newly opened, previously untested plate, one of these strips should be placed at the beginning of the plate and receive the full complement of kit controls.

   CAUTION: Use caution when assembling partial plates (mixing coated and uncoated) wells in a microplate. The OSP may not be able to differentiate between coated and uncoated (expired) wells and may produce results for any well position with an assigned ID number or control.

   CAUTION: Handle microwell strips with care. Do not touch the bottom exterior surface of the wells.

3. Assemble the microwell strips in the microwell strip holder, if necessary. **Microwell strips must be level in the microwell strip holder**. For incomplete plates, add white or uncoated microwell strips.

4. Prepare a record (plate map) identifying the placement of the controls and specimens in the microwells.

   Arrange the assay control wells so that well 1A is the substrate blank. From well 1A arrange all controls in a row (horizontal) or column (vertical) configuration as follows. Configuration is dependent upon software.

Well 1A	Substrate Blank
	Negative Control
	Negative Control
	Negative Control
	Positive Control
	Positive Control

5. **Verify that any manual dispensing equipment is set to deliver the specified volumes as stated in the procedure, following the equipment manufacturer's instructions.**
   Add controls and specimens to the microwells as follows.

   Sample Addition:

   a. Add 200 µL of Specimen Diluent to all wells, **except 1A** using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipetter-dilutor capable of delivering 200 µL with at least ± 5% accuracy.

   b. Add 10 µL of the control, or specimens to the appropriate wells using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipetter-dilutor capable of delivering 10 µL with at least ± 10% accuracy.

   c. If the controls and specimens have been manually delivered, to ensure the complete addition of control, or specimen, mix the sample and Specimen Diluent in the well by flushing the pipette tip several times.

   For Previously Diluted Sample Addition:

   a. Add 300 µL of Specimen Diluent to a tube or container.

   b. Add 15 µL of control or specimen to the tube. Mix thoroughly.

   c. Transfer 210 µL of each previously diluted control or specimen to the appropriate well position.

6. For manual processing of microwell plates, cover the microwell strip holder with a plate sealer. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing. Incubate at 37°C ± 1°C for **60 minutes ± 5 minutes**.

7. Level the strips in the microwell strip holder, if necessary. With an aspirator-washer device, aspirate and wash all wells **five** times with Wash Buffer (1X).

   CAUTION: Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. Follow the steps specified in order to ensure thorough washing.

   a. Aspirate the sample solutions from the microwells, then fill completely with Wash Buffer. Do not allow the wells to overflow. Allow approximately 20 seconds between the addition of Wash Buffer and subsequent aspiration.

   b. Complete the aspirate/fill sequence **four** additional times (5 times total).

   c. Completely aspirate wells. If processing manually, invert the plate and firmly tap on a clean paper towel to remove excess Wash Buffer, if necessary.

8. Add 200 µL of Conjugate to all wells, **except 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 200 µL with at least ± 5% accuracy.

9. For manual processing of microwell plates, cover the microwell strip holder with a **new, unused plate sealer**. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing. Incubate at 37°C ± 1°C for **60 minutes ± 5 minutes**.

10. Prepare **sufficient** Substrate Solution prior to use in Step 12 to allow time for OPD tablets to **dissolve completely**. Refer to **PREPARATION OF REAGENTS. Do not use more than a single preparation of Substrate Solution on a plate**.

11. After the second incubation, wash the wells as described in Step 7.

12. Add 200 µL of Substrate Solution to all wells, **including 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 200 µL with at least ± 5% accuracy. Incubate at room temperature (15 to 30°C) in the dark for **30 minutes ± 1 minute**.

13. Add 50 µL of 4N sulfuric acid (H_2SO_4) to all wells, **including 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 50 µL with at least ± 5% accuracy. To ensure proper mixing, acid should be added forcibly in a steady stream. If necessary, gently tap the plate to mix the contents. Care should be taken to avoid splashing of the contents of the microwells. When using an automated microplate processor, follow the instrument manufacturer's instructions with regard to mixing.

14. If necessary, wipe moisture from the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue before reading. If necessary, level the strips in the microwell strip holder. Read the microwell strip plate at a

wavelength of 490 nm or 492 nm. For dual wavelength readers, set the reference wavelength at 620 nm or 630 nm. Blank the reader on well 1A according to the instrument manufacturer's instructions.

For manual calculation, the user should ensure that the blank value (well 1A) has been subtracted from all control and specimen well values prior to applying the Quality Control criteria below.

NOTE: Microwell strip plates must be read within 60 minutes following the addition of 4N sulfuric acid (H_2SO_4). Plates must be stored in the dark until read.

Quality Control Procedures[14,15]

1. **Substrate Blank Acceptance Criteria**

   A plate is considered valid with respect to the substrate blank if the absorbance value of the substrate blank well (well 1A) is greater than or equal to -0.020 and less than or equal to 0.350. The plate is invalid if the substrate blank well is invalid.

2. **Negative Control Acceptance Criteria**

   a. Individual negative control values must be less than or equal to 0.120 and greater than or equal to -0.005. Numbers which are between 0.000 and -0.005 inclusive are valid and should be rounded to 0.000 for calculation. If one of the three control values is outside either of these limits, recalculate the negative control mean ($NC\bar{x}$) based upon the two acceptable control values. The plate is invalid and the test must be repeated if two or more of the three control values are outside either of the limits.

   b. Determine the mean of the negative control values ($NC\bar{x}$).
   Example:

Negative Control	Absorbance
1	0.010
2	0.030
3	0.020

Total Absorbance = 0.060

$$NC\bar{x} = \frac{\text{Total Absorbance}}{3} = 0.020$$

3. **Positive Control Acceptance Criteria**

   The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

   A plate is considered valid with respect to the positive control if both positive control values are greater than or equal to **0.800**, within the readable range of the microwell reader and do not differ by more than **0.600**.

   NOTE: Results beyond the upper limit of the readable range of the microwell reader may appear as "OVER" or "***" or ">".

4. **Calculation of the Cutoff Value**

   Cutoff Value = $NC\bar{x}$ + 0.600
   Example:

Negative Control	Absorbance
1	0.010
2	0.030
3	0.020

Total Absorbance = 0.060

$$NC\bar{x} = \frac{\text{Total Absorbance}}{3} = 0.020$$

Cutoff Value = 0.020 + 0.600 = 0.620

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than -0.080 should be retested in a single microwell. The specimen should be considered nonreactive if the retest absorbance value is less than the Cutoff Value, even if the retest absorbance value remains less than -0.080.
2. Specimens with absorbance values less than the Cutoff Value and greater than or equal to -0.080 are considered nonreactive. Further testing is not required.
3. Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive and should be retested in duplicate before final interpretation.
4. Upon retesting an initially reactive specimen, the specimen is considered repeatedly reactive for antibody to HCV if either or both duplicate determination(s) is (are) reactive, i.e., greater than or equal to the Cutoff Value.
5. After retesting an initially reactive specimen, the specimen is considered nonreactive for antibody to HCV if both duplicate determinations are negative, i.e., less than the Cutoff Value.

LIMITATIONS OF THE PROCEDURE

ORTHO® HCV Version 3.0 ELISA Test System is limited to the detection of anti-HCV in human serum or plasma. The presence of anti-HCV does not constitute a diagnosis of hepatitis C, but may be indicative of recent and/or past infection by hepatitis C virus. A nonreactive test result does not exclude the possibility of exposure to hepatitis C virus. Levels of anti-HCV may be undetectable in early infection.

Because the ORTHO® HCV Version 3.0 ELISA Test System was designed to screen individual units of blood or plasma, most data regarding its interpretation were derived from testing individual specimens. Insufficient data are available to interpret tests performed on other body fluids including pooled blood, or processed plasma and products made from such pools; testing of these specimens is not recommended.

Failure to add specimen or reagent may result in an erroneous result.

Data obtained from testing persons at increased risk or low risk for HCV infection suggest that repeatedly reactive specimens with high absorbance values are more likely to demonstrate the presence of anti-HCV in supplemental testing. Reactivity at

or slightly above the Cutoff Value is more frequently nonspecific, especially in specimens obtained from persons at low risk for infection. However, the presence of anti-HCV in some of these specimens can be demonstrated by supplemental testing.

The positive control in the test kit is not to be used to quantitate assay sensitivity. The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

When positive control values are beyond the linear range of the microwell reader, the positive control cannot be used to assess assay precision. Because true optical density values beyond the linear range are not known, differences between these values and values within the linear range cannot be quantitated.

EXPECTED RESULTS

In blood donor populations, the incidence of specimens found repeatedly reactive for anti-HCV by ORTHO® HCV Version 3.0 ELISA Test System has typically been less than 1%.

Reactivity in Blood Donors

The specificity of ORTHO® HCV Version 3.0 ELISA Test System in low-risk populations is based on a population of blood donors from four different test sites. A total of 33,025 specimens were tested. These consisted of 30,025 specimens from presumably healthy volunteer blood donors (sites 1, 2 and 3) and 3,000 specimens from commercial plasma donors (site 4). Rates of initial and repeat reactivities are shown in Table 1. Correlation between initial and repeat reactivity was 87.1%.

Table 1. Prevalence of Anti-HCV Reactivity in Blood Donors ORTHO® HCV Version 3.0 ELISA Test System

Site	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive
1	10,208	10,169	47 (0.46%)	39 (0.38%)
2	9,772	9,722	54 (0.55%)	50 (0.51%)
3	10,045	9,998	54 (0.54%)	47 (0.47%)
4	3,000	2,987	16 (0.53%)	13 (0.43%)
TOTAL	**33,025**	**32,876**	**171 (0.52%)**	**149 (0.45%)**

Specimens repeatedly reactive by ELISA were tested by supplemental strip immunoblot assay (SIA). Upon removal of specimens that were positive or indeterminate by SIA, specificity* in this low prevalence population was 99.95% (5 units per 10,000 donations).

*Specificity was calculated as follows:

Specificity = (TN/TN+FP)
Where TN = true negatives, that is, the number of specimens nonreactive by ELISA.
FP = false positives, that is, repeatedly reactive by ELISA and negative by SIA.

Detection of Anti-HCV Seroconversion in Transfusion Recipients with Transfusion-Associated NANBH (TA-NANBH)

Serial specimens collected from 21 patients with clinically documented TA-NANBH were tested at clinical (patient) site 2 by both ORTHO® HCV Version 3.0 ELISA Test System and ORTHO® HCV 2.0 ELISA Test System. HCV Version 3.0 detected seroconversion to anti-HCV in earlier bleeds in 5/21 (24%) patients. HCV 2.0 detected seroconversion to anti-HCV in an earlier bleed in 1/21 (5%) patients. In 15 patients, HCV Version 3.0 and HCV 2.0 detected anti-HCV in the same serial bleed.

Table 2. Difference in Detection of Anti-HCV in 21 TA-NANBH Patients

Patient Number	Earlier Detection by HCV ELISA	Difference in Detection Bleeds (Days)
1	HCV Version 3.0	2 (20)
2	HCV Version 3.0	1 (34)
3	HCV Version 3.0	1 (27)
4	HCV Version 3.0	2 (20)
5	HCV Version 3.0	2 (29)
6	HCV 2.0	1 (35)

There was no difference in the time to detection of anti-HCV ELISA reactivity in the remaining 15 patients.

Reactivity in Patients with NANBH

NANBH patients from clinical (patient) site 3 were tested with ORTHO® HCV Version 3.0 ELISA Test System. Patients were divided into acute and chronic duration disease based on alanine aminotransferase (ALT) levels and patterns. The frequency of anti-HCV detection was 75% in acute duration and 88% in chronic duration (Table 3).

Table 3. Detection of Anti-HCV in Patients with NANBH ORTHO® HCV Version 3.0 ELISA Test System

Category	Number of Patients	Repeatedly Reactive (%)
Acute[a]	231	75.3
Chronic[b]	59	88.1

[a] Diagnostic criteria for acute NANBH included: new onset of a symptomatic illness including a peak ALT of greater than 500 IU/L; absence of serologic markers for acute hepatitis A and B and no evidence of toxic (drug) induced hepatitis.

[b] Diagnostic criteria for chronic NANBH included: a negative test for hepatitis B surface antigen and abnormal ALT activity for more than 6 months.

Reactivity in High-Risk Populations

Reactivity in populations at risk for acquiring/transmitting NANBH was studied at two clinical (patient) sites. A total of 602 specimens from high-risk patients were tested with ORTHO® HCV Version 3.0 ELISA Test System. Table 4 shows the prevalence of anti-HCV reactivity in three high-risk groups.

Table 4. Detection of Anti-HCV in High-Risk Populations
ORTHO® HCV Version 3.0 ELISA Test System

Risk Group	Number of Specimens	Repeatedly Reactive (%)
Hemophiliacs	302	74.5
IV Drug Abusers	200	94.0
Renal Dialysis Patients	100	19.0

SPECIFIC PERFORMANCE CHARACTERISTICS

Reproducibility

The interassay and intra-assay reproducibility of ORTHO® HCV Version 3.0 ELISA Test System was assessed with a nonreactive specimen, a weakly reactive specimen (approximately 1.3 times the Cutoff Value), and a strongly reactive specimen (approximately 3.0 times the Cutoff Value). The specimens were tested with three different kit lots at two different test sites. Mean optical density (OD), standard deviation (SD) and coefficient of variation (%CV) results are shown in Table 5.

Table 5. Reproducibility of ORTHO® HCV Version 3.0 ELISA Test System

	Nonreactive (Mean OD = 0.042)		Weakly Reactive (Mean OD = 0.773)		Strongly Reactive (Mean OD = 1.857)	
Parameter Measured	SD	%CV	SD	%CV	SD	%CV
Inter-assay	0.003	NA*	0.079	10.3	0.168	9.0
Intra-assay	0.026	NA*	0.086	11.1	0.158	8.5

*%CVs are not meaningful when ODs approach zero.

Reproducibility (Migration Studies) on OSS with the ORTHO VERSEIA® Pipetter

The intra-assay (within plate) and inter-assay (between plate) reproducibility of the ORTHO® HCV Version 3.0 ELISA Test System was evaluated using an eight-member reproducibility panel. The reproducibility panel consisted of six members near the assay cutoff, one nonreactive member and one moderate to highly reactive member. Testing was conducted at 3 US Blood Centers and one internal site with one kit lot. Studies evaluated the reproducibility of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). On each pipetter, ten replicates each of the eight-member panel were tested on one plate, two times per day, for 5 days. Internal testing included 3 ORTHO® Summit Pipetters and 3 ORTHO VERSEIA® Pipetters; external testing included 3 ORTHO® Summit Pipetters and 3 ORTHO VERSEIA® Pipetters (one each per site).

Mean signal to cutoff (S/CO), standard deviation (SD), and coefficient of variation (CV%) results are presented in Table 6 for the two pipetting methods.

Table 6. Reproducibility Panel Testing: ORTHO® Summit System (OSS) [ORTHO® Summit Pipetter* and ORTHO VERSEIA® Pipetter, ORTHO® Summit Processor (OSP), and OAS]

				Inter-Plate**		Intra-Plate†		Total‡	
Platform	Panel Member	N	Mean S/CO Ratio	SD	CV (%)	SD	CV (%)	SD	CV (%)
ORTHO® Summit Pipetter	A	599	1.173	0.076	6.5	0.091	7.7	0.139	11.8
	B	600	0.991	0.078	7.9	0.072	7.3	0.119	12.0
	C	600	1.044	0.097	9.3	0.080	7.7	0.127	12.2
	D	599	0.887	0.042	4.7	0.057	6.4	0.086	9.7
	E	600	0.992	0.077	7.8	0.077	7.8	0.116	11.7
	F	600	3.040	0.180	5.9	0.184	6.1	0.290	9.5
	G	600	1.586	0.140	8.8	0.111	7.0	0.198	12.5
	H	600	0.085	0.006	6.6	0.014	16.3	0.016	18.9
ORTHO VERSEIA® Pipetter	A	595	1.324	0.080	6.1	0.077	5.8	0.143	10.8
	B	595	1.211	0.100	8.2	0.105	8.7	0.205	16.9
	C	596	1.290	0.156	12.1	0.130	10.1	0.286	22.2
	D	598	1.093	0.122	11.2	0.090	8.2	0.200	18.3
	E	599	1.133	0.104	9.2	0.078	6.9	0.212	18.7
	F	598	3.104	0.148	4.8	0.146	4.7	0.245	7.9
	G	595	1.645	0.123	7.5	0.109	6.6	0.186	11.3
	H	596	0.098	0.008	8.7	0.020	20.4	0.024	24.2

*The ORTHO® Summit Sample Handling System is now considered a legacy device and is no longer available for marketing.

**Inter-plate/Between Plate: Between run (Pipetter (Site) x Day): Variability of the assay performance from plate to plate, nested within pipetters and day, with pipetters nested within site

†Intra-plate/Within Plate: Between replicate: Variability of the assay performance from replicate to replicate.

‡Total: Sum of the individual components of variance including (1) Inter-plate, (2) Intra-plate, (3) Site to Site, (4) Day to Day, and (5) Pipetter to Pipetter variation.

Specificity

A total of 33,025 specimens from blood donors were tested, 32,876 of which were nonreactive. Upon removal of specimens positive or indeterminate in supplemental testing by SIA, specificity of ORTHO® HCV Version 3.0 ELISA Test System in this low prevalence population was 99.95%.

Specificity (Migration Studies) on OSS with the ORTHO VERSEIA® Pipetter

Comparison studies with 420 serum/plasma specimens known to be negative for HCV antibody were performed at 3 US Blood Centers and one internal site. Studies evaluated the specificity of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). These studies demonstrated that assay results are acceptable with the ORTHO® HCV Version 3.0 ELISA Test System using either method of pipetting.

Sensitivity

Sensitivity of ORTHO® HCV Version 3.0 ELISA Test System was evaluated in two studies:

The time to detect anti-HCV seroconversion in TA-NANBH patients (compared to ORTHO® HCV 2.0 ELISA Test System). See Table 2.

The frequency of detection of anti-HCV repeatedly reactive results in a NANBH patient population. See Table 3.

Sensitivity (Migration Studies) on OSS with the ORTHO VERSEIA® Pipetter

Comparison studies with 110 serum/plasma specimens known to be positive for HCV antibody were performed at 3 US Blood Centers and one internal site. Studies evaluated the sensitivity of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). These studies demonstrated that assay results are acceptable with the ORTHO® HCV Version 3.0 ELISA Test System using either method of pipetting.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Reproducibility of ORTHO® HCV Version 3.0 ELISA Test System was assessed using 20 cadaveric and 20 normal donor sera. These specimens were spiked with anti-HCV positive plasma to give reactivity near the assay cutoff. Each of the specimens was tested once on six different days on each of three lots of ORTHO® HCV Version 3.0 ELISA Test System at one site. Reproducibility testing was performed by both manual and automated processing methods. For each processing method, cadaveric and living donor specimens were 100% reactive across kit lots and the %CVs were comparable for both specimen groups.

Kit Lot 1

		Number of Donors	Replicates	% Positive	Mean S/C	CV(%)
Manual	Cadaveric	20	120	100	1.828	20.0
	Living Donor	20	120	100	1.706	11.8
Automated	Cadaveric	20	120	100	1.961	16.0
	Living Donor	20	120	100	1.949	10.7

Kit Lot 2

		Number of Donors	Replicates	% Positive	Mean S/C	CV(%)
Manual	Cadaveric	20	120	100	1.981	17.2
	Living Donor	20	120	100	1.851	13.1
Automated	Cadaveric	20	120	100	2.086	14.3
	Living Donor	20	120	100	2.102	11.5

Kit Lot 3

		Number of Donors	Replicates	% Positive	Mean S/C	CV(%)
Manual	Cadaveric	20	120	100	2.070	17.7
	Living Donor	20	120	100	1.943	13.4
Automated	Cadaveric	20	120	100	2.101	17.5
	Living Donor	20	120	100	2.160	14.0

Specificity

Specificity was evaluated using 50 cadaveric specimens collected up to 23.7 hours after death and 50 normal donor specimens. Testing was performed across three lots of ORTHO® HCV Version 3.0 ELISA Test System by both manual and automated processing methods. For the manual method, the mean signal to cutoff (S/C) ratio was 0.071 for the cadaveric specimens, and the mean S/C ratio was 0.030 for the normal donor specimens. For the automated method, the mean signal to cutoff (S/C) ratio was 0.064 for the cadaveric specimens, and the mean S/C ratio was 0.042 for the normal donor specimens. The results are presented in Table 7.

Table 7. Reactivity in the ORTHO® HCV Version 3.0 ELISA Test System

Population	Number of Specimens	Mean S/C	Nonreactive	Initially Reactive
Manual Processing				
Cadaveric	50	0.071	50 (100.0%)	0 (0.0%)
Normal Donor	50	0.030	50 (100.0%)	0 (0.0%)
Automated Processing				
Cadaveric	50	0.064	50 (100.0%)	0 (0.0%)
Normal Donor	50	0.042	50 (100.0%)	0 (0.0%)

The ORTHO® HCV Version 3.0 ELISA Test System has an estimated specificity in cadaveric specimens of 100.0% (50/50) with a 95% exact confidence interval of 92.9% to 100.0%.

Sensitivity

Sensitivity was evaluated using 50 cadaveric specimens collected up to 23.7 hours after death and 50 normal donor specimens. All specimens were pre-screened for anti-HCV and were found to be nonreactive. All specimens were spiked with anti-HCV positive plasma to give reactivity near the assay cutoff. Testing was performed approximately 47 hours after spiking using three lots of ORTHO® HCV Version 3.0 ELISA Test System by both manual and automated processing methods. Since the specimens were spiked to be reactive, duplicate repeat testing was not performed for initially reactive specimens. For the manual method, the mean signal to cutoff (S/C) ratio was 1.904 for the cadaveric specimens, and the mean S/C ratio was 1.893 for the normal donor specimens. The calculated difference between the cadaveric specimens and the normal donor specimens tested by the manual method was 0.011 S/C, which was determined by the F-test not to be statistically significant (p=0.866). For the automated method, the mean signal to cutoff (S/C) ratio was 2.136 for the cadaveric specimens, and the mean S/C ratio was 2.362 for the normal donor specimens. The calculated difference between the cadaveric specimens and the normal donor specimens tested by the automated method was 0.226 S/C, which was determined by the F-test to be statistically significant (p=0.0003). However, all results for the cadaveric and normal donor specimens were reactive with the ORTHO® HCV Version 3.0 ELISA Test System resulting in 100.0% reactivity. The results are presented in Table 8.

Table 8. Reactivity in the ORTHO® HCV Version 3.0 ELISA Test System

Population	Number of Specimens	Mean S/C	Nonreactive	Initially Reactive
Manual Processing				
Cadaveric	50	1.904	0 (0.0%)	50 (100.0%)
Normal Donor	50	1.893	0 (0.0%)	50 (100.0%)
Automated Processing				
Cadaveric	50	2.136	0 (0.0%)	50 (100.0%)
Normal Donor	50	2.362	0 (0.0%)	50 (100.0%)

The ORTHO® HCV Version 3.0 ELISA Test System has an estimated sensitivity in spiked cadaveric specimens of 100.0% (50/50) with a 95% exact confidence interval of 92.9% to 100.0%.

SUMMARY OF REVISIONS

Section	Revision
REAGENTS	Removed EDTA from the list of preservatives.
PRECAUTIONS	Clarified the materials contained in the OPD tablet. Updated the hazardous material statements for desiccant.
Back Page	Updated copyright information.

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KEY TO SYMBOLS / LÉGENDE DES SYMBOLES / CLAVE DE LOS SÍMBOLOS

The following symbols may have been used in the labeling of this product. / Les symboles suivants ont pu être utilisés sur l'étiquette de ce produit. / Los siguientes símbolos pueden haber sido empleados en el etiquetado de este producto.

Do Not Reuse / Ne pas réutiliser / No reutilizar

Use by or Expiration Date (Year-Month-Day) / À utiliser avant la date de péremption (année-mois-jour) / Usar antes de o Fecha de caducidad (año-mes-día)

Lot Number / Numéro de lot / Número de lote

Serial Number / Numéro de série / Número de serie

Catalog Number or Product Code / Référence catalogue ou code produit / Referencia de catálogo o Código del producto

Attention: See Instructions for Use / Attention : consulter le feuillet technique / Atención: Consultar las instrucciones de uso

Manufacturer / Fabricant / Fabricante

Authorized Representative in the European Community / Mandataire dans l'Union européenne / Representante autorizado en la Unión Europea

Contains Sufficient for "n" Tests / Suffisant pour << n >> dosages / Contiene suficiente para "n" ensayos

In vitro Diagnostic Medical Device / Pour diagnostic in vitro / Producto sanitario para diagnóstico in vitro

Upper Limit of Temperature / Conserver à une température égale ou inférieure à / Límite superior de temperatura

Lower Limit of Temperature / Conserver à une température égale ou supérieure à / Límite inferior de temperatura

Temperature Limitation / Conserver à une température comprise entre / Limitación de temperatura

Consult Instructions for Use, "n" Version / Consultez le feuillet technique << n >> version / Atención: ver las instrucciones de uso "n" versión

Biological Risks / Risques biologiques / Riesgos biológicos

Do not use if damaged / Ne pas utiliser si endommagé / No usar si está dañado

Health Hazards / Dangereux pour la santé / Riesgos para la salud

Acute Toxicity / Toxique ou mortel / Toxicidad aguda

Serious Health Hazards / Très dangereux pour la santé / Riesgos graves para la salud

Corrosive / Corrosif / Corrosivo

KEY TO SYMBOLS / LÉGENDE DES SYMBOLES / CLAVE DE LOS SÍMBOLOS

Continued / Suite / Continuación

Environmental or Aquatic Toxicity / Dangereux pour l'environnement aquatique / Toxicidad marina o medioambiental

Fragile, Handle with Care / Attention, fragile / Frágil; manipular con cuidado

Keep Dry / Tenir au sec / Mantener seco

This end up / Haut / Este lado hacía arriba

CONTROL +

Positive Control / Contrôle positif / Control positivo

CONTROL −

Negative Control / Contrôle négatif / Control negativo

CALIBRATOR +

Positive Calibrator / Calibrateur positif / Calibrador positivo

CALIBRATOR −

Negative Calibrator / Calibrateur négatif / Calibrador negativo

Confirmatory Control

Confirmatory Control / Contrôle de confirmation / Control de confirmación

Recombinant Antigens Provided by

Recombinant Antigens Provided by / Antigènes recombinants fournis par / Antígenos recombinantes suministrados por

Antibody to Hepatitis B Surface Antigen

Antibody to Hepatitis B Surface Antigen / Anticorps dirigé contre l'antigène de surface du virus de l'hépatite B / Anticuerpo frente al antígeno de superficie de la hepatitis B

Antibody to Hepatitis B Surface Antigen: Peroxidase Conjugate Concentrate

Antibody to Hepatitis B Surface Antigen: Peroxidase Conjugate Concentrate / Anticorps dirigé contre l'antigène de surface du virus de l'hépatite B : conjugué concentré à la peroxydase / Anticuerpo frente al antígeno de superficie de la hepatitis B: concentrado de conjugado a peroxidasa

Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations. / Der Grüne Punkt (Point Vert). Le fabricant suit certaines règles de mise au rebut pour les déchets des matériaux d'emballage / Punto Verde (der grüne Punkt). El fabricante sigue la regulación sobre gestión de residuos de los embalajes

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