HEPATITIS C VIRUS ENCODED ANTIGEN (RECOMBINANT c100-3, HC-31, AND HC-34) ABBOTT HCV EIA 2.0

NOTE CHANGES HIGHLIGHTED

ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETECTION OF ANTIBODY TO HEPATITIS C VIRUS (ANTI-HCV) IN HUMAN SERUM, PLASMA, OR CADAVERIC SERUM.

69-4837/R14

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CUSTOMER SUPPORT CENTER
1-800-323-9100
U.S. License No. 43

Recombinant Antigens Provided by:
Chiron Corporation
Emeryville, CA 94608

List No. 4A14
Printed in U.S.A.

NAME AND INTENDED USE

ABBOTT HCV EIA 2.0 is an *in vitro* Enzyme Immunoassay for the Qualitative Detection of Antibody to Hepatitis C Virus (anti-HCV) in Human Serum, Plasma, or Cadaveric Serum.

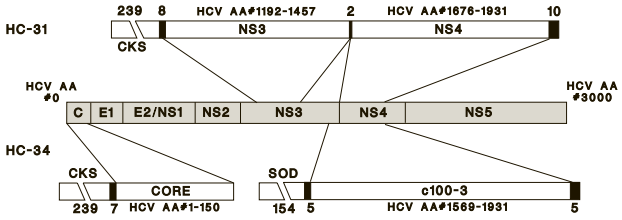
SUMMARY AND EXPLANATION OF TEST

The technology for enzyme immunoassays (EIA) was first described in the early 1970's.[1] The development of the technology for detection of viral infections has closely followed the progress of viral research in the identification of antigens and antibodies related to infection. Solid phase EIAs utilize antigens or antibodies coated on a surface. A series of antigen and antibody reactions follows with the penultimate reaction involving an enzyme labeled antibody or antigen that is capable of yielding a colored end product. The enzyme reaction is stopped with acid and the amount of colored end product, which is quantified as a measure of the amount of antigen or antibody bound at an earlier step, is read with a spectrophotometer. The ABBOTT HCV EIA 2.0 is an enzyme immunoassay which has HCV recombinant antigens bound to the solid phase and is designed to detect anti-HCV antibodies.

Implementation of sensitive testing for hepatitis B virus (HBV) in the early 1970's reduced the incidence of post-transfusion hepatitis (PTH). However, it was quickly recognized that the majority of PTH which remained was caused by an as yet unidentified virus.[2-4] The term non-A, non-B hepatitis (NANBH) has been used to describe hepatitis in patients who do not develop antibodies to hepatitis A virus (HAV), HBV, cytomegalovirus or Epstein-Barr virus and do not have a clinical history of other potential causes of hepatitis.[5]

Additional studies indicated that the agent(s) of NANBH caused 90% of PTH and was transmitted primarily by the percutaneous route. Other studies indicated that 1-7% of healthy donors may be infective and that there was a correlation between the presence of elevated serum alanine aminotransaminase (ALT) levels and antibodies to HBV core antigen (anti-HBc) in the donor and the risk of NANBH developing in the recipient.[6-11] The cloning segments of an agent, designated hepatitis C virus (HCV), from the plasma of a chronic NANBH chimpanzee led to the development of a recombinant nonstructural antigen (c100-3) which was used in an assay to identify antibodies to HCV (anti-HCV).[12] Tests using c100-3 detect anti-HCV in 0.2-1.2% of random blood donors (U.S.A., Europe and Japan), 15-25% of acute NANBH patients and 67-85% of chronic NANBH patients.[13-19]

Several lines of evidence suggest that the single-antigen assays for detection of anti-HCV antibodies do not identify all HCV infected individuals.[20] The ABBOTT HCV EIA 2.0 has been developed to detect antibodies to proteins expressed by putative structural (HC-34) and nonstructural (HC-31, c100-3) regions of the HCV genome. A diagram of the putative HCV genome and the position of the recombinant antigens c100-3, HC-31 and HC-34 is represented below. Individual blood or plasma donors or patients (based on clinical evaluation) may be tested for antibodies to HCV. The presence of these antibodies indicates that the individual (donor or patient) has been infected with HCV, may harbor infectious HCV, and may be capable of transmitting NANBH.



THE RECOMBINANT HCV PROTEINS USED IN ABBOTT HCV EIA 2.0

HC-34 A recombinant HCV protein expressed in *Escherichia coli* (*E. coli*) contains HCV[21] amino acids #1 to 150 presumed to encode core structural region. HC-34 is a chimeric fusion protein with 239 amino acids of *E. coli* protein CMP-KDO synthetase (CKS),[22,23] 7 linker amino acids and HCV amino acids #1 to 150.

HC-31 A recombinant HCV protein expressed in *E. coli* contains HCV amino acids #1192-1457 presumed to encode nonstructural protein 3 (NS3) and HCV amino acids #1676-1931 presumed to encode nonstructural protein 4 (NS4). HC-31 is a chimeric fusion protein with 239 amino acids of *E. coli* CKS, 8 linker amino acids, HCV amino acids #1192-1457, HCV amino acids #1676-1931 and 10 linker amino acids at the carboxy terminus.

c100-3 A recombinant HCV protein expressed in yeast contains HCV amino acids #1569-1931 from NS3/NS4 region. c100-3 is a fusion protein with 154 amino acids of superoxide dismutase (SOD),[12] 5 linker amino acids, HCV amino acids #1569-1931 and 5 linker amino acids at the carboxy terminus.

Hepatitis C antigens c100-3 and HC-34 are prepared under U.S. license by Chiron Corporation under shared manufacturing agreement. Recombinant antigen HC-31 is prepared by Abbott Laboratories. The ABBOTT HCV EIA 2.0 test kit is manufactured and sold under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

In the ABBOTT HCV EIA 2.0, human serum, plasma, or cadaveric serum is diluted in a specimen diluent and incubated with a polystyrene bead coated with recombinant HCV antigens c100-3, HC-31 and HC-34. If antibody is present in the sample, immunoglobulins in the patient sample are affixed to the coated bead. After removing the unbound materials and washing of the bead, human immunoglobulins remaining bound to the solid phase are detected by incubating the bead-antigen-antibody complex with a solution containing horseradish peroxidase labeled goat antibodies directed against human immunoglobulins.

Unbound enzyme conjugate is then removed and the beads are washed. Next, o-Phenylenediamine•2 HCl (OPD) solution containing hydrogen peroxide is added to the bead. In the presence of hydrogen peroxide, the peroxidase enzyme which is labeled to the bound conjugate is oxidized. The oxidized peroxidase then reacts with the o-Phenylenediamine•2 HCl (OPD) resulting in the reduction of peroxidase and the oxidation of OPD. The amount of oxidized OPD, which has an orange-yellow color, is proportional to the amount of anti-HCV which is bound to the bead. The enzyme reaction is stopped by the addition of 1*N* Sulfuric Acid and the intensity of color developed is read using a spectrophotometer set at 492nm.

REAGENTS

ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

① 100/1000/5000 Hepatitis C Virus Encoded Antigen (Recombinant c100-3, HC-31 and HC-34) Coated Beads.

Recombinant Protein	Host Organism	Fusion Protein
HC-31	*E. coli*	CKS*
HC-34	*E. coli*	CKS*
c100-3	*S. cerevisiae*	SOD**

* CMP-KDO Synthetase

** Superoxide Dismutase

② 3 Vials (1 mL each)/3 Vials (5 mL each)/15 Vials (5 mL each) Conjugate Concentrate. Goat Antibody to Human IgG (H + L): Peroxidase (Horseradish). Minimum Concentration: 0.02 µg/mL in TRIS Buffer with Animal Serum (Calf) and red dye number 33. Preservatives: 0.01% Gentamicin and 0.01% Thimerosal.

③ 3 Vials (19 mL each)/3 Vials (95 mL each)/15 Vials (95 mL each) Conjugate Diluent containing 20% Animal Sera (Goat, Calf) in TRIS Buffer. Preservatives: 0.01% Gentamicin and 0.01% Thimerosal.

④ 1 Vial (2 mL)/2 Vials (2 mL each)/10 Vials (2 mL each) Positive Control. Inactivated Human Plasma Reactive for anti-HCV and nonreactive for HBsAg, HIV-1 Ag and anti-HIV-1/HIV-2. Minimum Titer: 1:2. Preservative: 0.1% Sodium Azide.

⑤ 1 Vial (2 mL)/2 Vials (2 mL each)/10 Vials (2 mL each) Negative Control. Human Plasma is nonreactive for HBsAg, HIV-1 Ag, anti-HCV and anti-HIV-1/HIV-2. Preservative: 0.1% Sodium Azide.

⑥ 2 Vials (20 mL each)/4 Vials (100 mL each)/20 Vials (100 mL each) Specimen Diluent containing TRIS Buffer, 0.2% Triton X-100, Protein Lysates and Animal Sera (Goat, Calf). Preservative: 0.1% Sodium Azide.

* There are no components 7 and 8.

⑨ 1 Bottle (10 tablets)/2 Bottles (40 tablets each)/10 Bottles (40 tablets each) OPD (o-Phenylenediamine•2 HCl) Tablets. OPD/Tablet: 12.8 mg.

⑩ 1 Bottle (55 mL)/2 Bottles (220 mL each)/10 Bottles (220 mL each) Diluent for OPD (o-Phenylenediamine•2 HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

The stopping reagent is provided as an accessory to the ABBOTT HCV EIA 2.0 kit and consists of:

⑪ 1*N* Sulfuric Acid, No. 7212. (Most U.S. and International locations).

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, Sulfuric Acid must pass the following test each time it is prepared. The following test cannot be performed on the COMMANDER® system. Use a Quantum™II to perform this test.

1. Pipette 300 µL of OPD Substrate Solution into 5 EIA reaction tubes or acid washed/distilled or deionized water rinsed tubes.
2. Add 1 mL of the 1*N* Sulfuric Acid under test to each of the five tubes.
3. Measure the A_{492} of the OPD/Acid Solution against distilled or deionized water at "0 TIME" and "120 MIN".
4. Calculate the Mean Absorbance at "0 TIME" and "120 MIN".
5. To be acceptable, acid must exhibit:
   a. an A_{492} of less than 0.040 at "0 TIME" *and*
   b. a difference of less than 0.030 units in the values obtained at "0 TIME" and "120 MIN".

Additional Reagents available (Most International Locations):

- 1*N* Sulfuric Acid, No. 7212-01 (110 mL).
- 6*N* Sulfuric Acid, No. 7212-03 (110 mL).

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

ABBOTT HCV EIA 2.0 MEETS FDA POTENCY REQUIREMENTS.

Safety Precautions

CAUTION:

This product contains human sourced and/or potentially infectious components. Some components sourced from human blood have been tested and found to be reactive for anti-HCV by FDA licensed tests. Refer to Reagents Section for details. The positive control has been inactivated by heat treatment. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[24] Biosafety Level 2[25] or other appropriate biosafety practices[26,27] should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where these materials are handled.
4. Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.[28,29]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.[30,31]

This product contains Sodium Azide as a preservative in the Positive Control, Negative Control and Specimen Diluent. Sodium Azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of solutions containing Sodium Azide.

To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health (U.S.A.) recommends the following:

1) Siphon liquid from trap using a rubber or plastic hose.
2) Fill with 10% sodium hydroxide solution.
3) Allow to stand for 16 hours.
4) Flush well with water.

Some components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R22 Harmful if swallowed.
R32 Contact with acids liberates very toxic gas.
S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36 Wear suitable protective clothing.
S46 If swallowed, seek medical advice immediately and show this container or label.

The OPD tablets listed in the **REAGENTS** section of this package insert contain o-Phenylenediamine•2 HCl and Sodium Carbonate. The OPD tablets are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R40/22 Harmful: possible risks of irreversible effects if swallowed.
R36/38 Irritating to eyes and skin.
R43 May cause sensitization by skin contact.
S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S46 If swallowed, seek medical advice immediately and show this container or label.

The 6*N* Sulfuric Acid listed in the **REAGENTS** section of this package insert is classified per applicable European Community (EC) Directives as: Corrosive (C). The following are the appropriate Risk (R) and Safety (S) phrases.

R35 Causes severe burns.
S1/2 Keep locked up and out of the reach of children.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately.

Handling Precautions

1. Do not use kit beyond the expiration date.
2. Do not mix reagents from different lots.

   NOTE: Any OPD reagent lot or acid lot may be used with any ABBOTT EIA Reagent kit.
3. Avoid microbial contamination of specimens, reagents and water used for washing. Use of disposable pipette tips is recommended. Avoid chemical contamination of reagents and equipment.
4. Do not expose OPD reagents to strong light during storage or incubation.
5. Avoid contact of the OPD Substrate Solution and 1*N* Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1*N* Sulfuric Acid to come in contact with any metal parts. Prior to use, rinse glassware used with OPD Substrate Solution thoroughly with 1*N* acid (sulfuric or hydrochloric) using approximately 10% of the container volume followed by three washes of distilled water at the same volume.
6. If the desiccant obstructs the flow of beads, remove desiccant prior to dispensing beads. Replace desiccant in bead bottle, and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle.
7. Use a clean, dedicated dispenser for diluted conjugate to avoid neutralization.
8. The Negative and Positive Controls, as provided, should be dispensed and diluted in the same manner as specimens.
9. Ensure that specimen is added to reaction well. If a specimen is inadvertently not added, assay may yield an erroneous, nonreactive result.
10. Inadequate adherence to package insert instructions may result in erroneous results.
11. Use accurately calibrated equipment.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (15 to 30°C).

CAUTION: Do not open OPD Tablet bottle until it is at room temperature.

Five to ten minutes prior to Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine•2 HCl) Tablet in Diluent for OPD. DO NOT USE A TABLET THAT IS NOT INTACT.

Using clean pipettes and metal free containers (such as plastic ware or acid-washed and distilled water-rinsed glassware) follow the procedure below:

1. Transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using nonmetallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet, and close bottle tightly. Allow tablet to dissolve. Do not cap or stopper the Substrate Solution bottle while the tablets are dissolving.

NOTE: The OPD Substrate Solution **must** be dispensed within 60 minutes of preparation and **must not** be exposed to strong light. Record the preparation time and expiration time of the OPD Substrate Solution on the container.

3. Just prior to dispensing for Color Development, swirl gently to obtain a homogeneous solution. Remove air bubbles from dispenser tubing, and prime dispenser prior to use.

OPD PREPARATION CHART

No. Beads	Tablets	Diluent
13	1	5 mL
28	2	10 mL
43	3	15 mL
58	4	20 mL
73	5	25 mL
88	6	30 mL
103	7	35 mL
118	8	40 mL
133	9	45 mL
148	10	50 mL

NOTE: **300 µL of OPD Substrate Solution is required for each specimen or Control as well as for each substrate blank.** Laboratories using the COMMANDER Parallel Processing Center (PPC™) will require approximately an additional 3 mL of OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

1. Store kit reagents at 2 to 8°C. OPD Tablets and 1*N* Sulfuric Acid may be stored at 2 to 30°C. Do not freeze kit reagents.
2. Bring all reagents to room temperature (15 to 30°C) before use (approximately 30 minutes) and return to storage conditions indicated above immediately after use.

CAUTION: Do not open Bead bottle or OPD Tablet bottle until it is at room temperature.

3. Retain desiccant bags in Bead bottle and OPD Tablet bottle at all times during storage.
4. Reconstituted OPD Substrate Solution **MUST** be stored at room temperature and **MUST** be dispensed within 60 minutes of preparation. Do not expose to strong light.

Indication of Instability or Deterioration of Reagents

The OPD Substrate Solution (OPD plus Diluent for OPD) should be colorless to pale yellow. A yellow-orange color of the Solution indicates that the reagent has been contaminated and must be discarded.

A value of less than 0.400 absorbance units for the difference between the Positive and Negative Control Means (P-N) may indicate technique errors or deterioration of the kit or OPD reagents. Such runs must be repeated.

SPECIMEN COLLECTION AND PREPARATION

ABBOTT HCV EIA 2.0 may be performed on human serum, plasma, or cadaveric serum.

1. Either serum (including serum collected in serum separator tubes), plasma (collected in EDTA, potassium oxalate, heparin, or citrate based anticoagulants), or cadaveric serum may be used in the ABBOTT HCV EIA 2.0 assay. The correct ratio of anticoagulant quantity to specimen volume as recommended by the manufacturer of anticoagulant is required.
2. Performance has not been established using body fluids (e.g., urine, saliva, or pleural fluid) other than serum, plasma, or cadaveric serum.
3. Do not use heat-inactivated specimens.
4. When possible, clear, non-hemolyzed specimens should be used. Specimens containing precipitate may give inconsistent test results. Such specimens should be clarified prior to testing.
5. Remove serum or plasma from the clot or red blood cells as soon as possible to avoid hemolysis.
6. Serum or plasma specimens may be stored for up to 14 days at 2 to 8°C. However, if storage periods greater than 14 days are anticipated, the specimens should be stored frozen at -10°C or colder.
   NOTE: Do not freeze specimens on the clot or on the red blood cells.
7. Cadaveric serum specimens may be stored for up to five days at 2 to 8°C. However, if storage periods greater than five days are anticipated, the specimens should be stored frozen at -20°C or colder.
   NOTE: Do not freeze specimens on the clot or on the red blood cells.
8. Ten negative and ten weakly reactive specimens showed no qualitative performance differences when subjected to five freeze-thaw cycles, however, multiple freeze-thaw cycles should be avoided, if possible. Specimens must be mixed thoroughly after thawing prior to testing.
9. For cadaveric serum specimens, no performance differences were observed in ten low-level reactive specimens subject to three freeze-thaw cycles, however, multiple freeze-thaw cycles should be avoided. Specimens must be mixed thoroughly after thawing prior to testing.
10. No qualitative performance differences were observed when ten negative and ten weakly reactive specimens were tested with elevated levels of bilirubin (7.4-27.7 mg/dL), hemoglobin (300-1000 mg/dL), or triglycerides (218.8-560.3 mg/dL).
11. For cadaveric serum specimens, no performance differences were observed in ten negative and ten low-level reactive specimens both tested with or without elevated levels of bilirubin (≤20 mg/dL), hemoglobin (≤500 mg/dL), or triglycerides (≤3000 mg/dL).
12. If serum or plasma specimens are to be shipped, they should be packaged and labeled in compliance with federal and international regulations covering the transportation of clinical specimens and etiologic agents.[32] Specimens may be shipped ambient, refrigerated (2 to 8°C) on wet ice, or frozen (-10°C or colder) on dry ice. Upon arrival, if specimens are to be stored, they may be stored at 2 to 8°C for up to 14 days after collection or frozen (-10°C or colder).
13. If cadaveric serum specimens are to be shipped, they should be packaged and labeled in compliance with federal and international regulations covering the transportation of clinical specimens and etiologic agents.[32] Cadaveric serum specimens may be shipped refrigerated (2 to 8°C) on wet ice or frozen (-10°C or colder) on dry ice. Upon arrival, if specimens are to be stored, they may be stored at 2 to 8°C for up to five days after collection or frozen (-10°C or colder).

PROCEDURE

Materials Provided

No. 4A14 ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

(See **REAGENTS** for a complete listing)

The list of accessories required for the COMMANDER System is found in the COMMANDER Operations Manual(s). A combination of accessories is included with the COMMANDER System. The ABBOTT HCV EIA 2.0 is designed to be compatible with the COMMANDER System and the Quantum II.

An optimum combination of the following accessories is provided:

- Reaction Trays
- Cover Seals
- Assay Tubes with Identifying Cartons

Materials Required but not Provided:

- Distilled or deionized water which is free from microbial contamination.
- 1*N* Sulfuric Acid, No. 7212 (Most U.S. and International locations).
- Precision pipettes and pipette tips or similar equipment to deliver 10 µL, 200 µL, 300 µL, 400 µL (tolerance is ± 5%) and 1 mL (tolerance is ± 10%).
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Metal free containers for the OPD Substrate Solution, can be plastic ware, or acid-washed, distilled water-rinsed glassware.
- QwikWash; or device for washing beads with a vacuum source and a double trap for retaining the aspirate and maintaining minimum vacuum of 21 inches of mercury to deliver a total wash volume of 11-18 mL per well.
- COMMANDER Dynamic Incubator or water bath capable of maintaining temperature at 40 ± 2°C.
- COMMANDER PPC or Quantum II.
- Bead Dispenser.
- Nonmetallic forceps.
- Membrane Seal Puncture Tool for acid bottles.
- OPD Tray Covers.
- Reagent Blanking Beads (for COMMANDER testing).
- Protective gloves.
- 5.25% sodium hypochlorite (household bleach).

Additional Reagents Available

1. ABBOTT OPD (o-Phenylenediamine•2 HCl) Reagent, No. 6172.
2. 1*N* Sulfuric Acid, No. 7212-01.
3. 6*N* Sulfuric Acid, No. 7212-03.

ABBOTT HCV EIA 2.0 PROCEDURE

PRELIMINARY COMMENTS

Laboratories using the COMMANDER System or Quantum II should refer to the appropriate manuals and note special instrument instructions below.

1. Assay three Negative Controls and three Positive Controls adjacent to the specimens using one bottle of diluted conjugate in the assay run. An assay run is defined as a minimum of three Negative Controls, three Positive Controls and one specimen on one 20- or 60-well tray or a maximum of three Negative Controls, three Positive Controls and 494 specimens on 20- or 60-well trays using a full bottle of diluted conjugate (500 tests). Use one preparation of working reagents per run.

   Ensure that all reaction trays containing Controls and/or specimens are subjected to the same process and incubation times. This requires maintenance of specific time intervals between processing trays. Once the assay has been started, all subsequent steps must be completed without interruption and within the recommended time limits. Precise timing of enzyme immunoassays is critical.

CAUTION: Use a separate disposable pipette tip for each specimen and Control in order to avoid cross-contamination.

2. Approximately 30 minutes prior to beginning the assay procedure, remove reagent vials from the kit and bring them to room temperature (15 to 30°C) and mix gently. Adjust COMMANDER Dynamic Incubator, water bath or equivalent to 40 ± 2°C.
3. Make certain that sufficient diluted conjugate is available for the test. If necessary, prepare additional conjugate reagent. (See **Preparation of Conjugate Reagent** section which follows.) Do not mix bottles of diluted conjugate reagent.
4. **Separate Negative and Positive Controls must be run with each bottle of diluted conjugate reagent.**
5. Identify the reaction tray wells for each Control and specimen.
6. After each step, visually verify the presence of solution and bead in each well.
7. **The exact order of specimen and reagent addition as described in this test procedure must be followed.**

Preparation of Conjugate Reagent

1. Bring Conjugate Concentrate and Conjugate Diluent to room temperature before mixing.
2. Carefully empty the contents of a Conjugate Concentrate vial (with red dye) into a vial of Conjugate Diluent. This can be done most efficiently by slowly squeezing the small vial 2 to 3 times while maintaining the nozzle within the opening of the large vial. Avoid foaming. One vial of diluted conjugate is sufficient for 100 tests (100 Test Kit) or 500 tests (1000 and 5000 Test Kit).
3. Reseal the large vial. Mix thoroughly by slowly inverting the vial several times. Do not vortex.
4. **Write the date of dilution and expiration date in the space provided on the conjugate diluent label. Conjugate is stable for 14 days (though not to exceed the kit expiration date) after dilution when stored at 2 to 8°C.**
5. Allow diluted conjugate to equilibrate at room temperature for approximately 60 minutes prior to use.
6. If storing the diluted conjugate, store at 2 to 8°C. Bring to room temperature before using.
7. Do not mix vials of diluted conjugate. Separate Negative and Positive Controls must be run with each vial of diluted conjugate.

PROCEDURAL NOTES

SAMPLE PIPETTING AND DILUTION

A. When using a manual method of sample dilution, follow the steps in the "Dilution of Specimen" section of the ASSAY PROCEDURE.

B. When pipetting with the COMMANDER Flexible Pipetting Center (FPC™), use System Software version 2.5 or higher. Use FPC Assay Update Diskette Version 2.5 or higher. Use the appropriate Assay Protocols as follows:
A-HCV 2.0 PPC D0 or A-HCV 2.0 PPC D1 for PPC processing, or
A-HCV 2.0 QT D0 or A-HCV 2.0 QT D1 for Quantum II processing.

ASSAY SELECTION ON THE PPC

A. Insert tray and select the appropriate assay number for -A-HCV EIA 2.0. An operator-edited version may be used if the edited lines are consistent with package insert specifications and are supported by documentation at the time of edit.
Follow the instructions on the instrument display board.
When using an automated pipetting device such as the COMMANDER Flexible Pipetting Center (FPC) verify that the correct PPC Assay Protocol has been selected for processing.

B. Verify reagent dispenser assignment:

Station	Reagent Dispenser	Volume
2	Conjugate	200 µL
4	OPD Solution	300 µL
5	Acid	300 µL

NOTE: Always prime dispensers to assure that the appropriate volume of reagent is dispensed. Verify dispenser accuracy per your Standard Operating Procedures.

C. If using a water bath, carefully wipe or blot the bottom of the tray dry before inserting it into the PPC.

D. BLANKING (PPC only)
NOTE: Use ABBOTT COMMANDER Reagent Blanking Beads only.
1. During the conjugate incubation step, prepare a "blanks" tray using a separate tray. Place one blanking bead into each of the five wells, A1 through A5.
2. At the conclusion of the conjugate incubation step, press the Blank key and insert the "blanks" tray, followed immediately by the first assay tray.
3. At the conclusion of the OPD incubation step, insert the "blanks" tray as the first tray of the batch.

GENERAL NOTES

1. The Negative and Positive Controls should be treated as specimens.
2. **Verify that dispensing equipment delivers specified volume and appropriate dilutions for each procedure.**
3. When dispensing beads, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells of the reaction tray as directed in the Single Bead Dispenser and Multi-Bead Dispenser inserts.
4. Do not splash liquid while tapping trays.
5. Make sure cover seals adhere tightly to all wells. Proper mixing of samples is required for accurate results. Tap trays to thoroughly mix contents and beads. The beads should move within the well during the tapping process.
6. When washing beads, follow the directions provided with your washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.

COMMANDER DYNAMIC INCUBATOR

When using the COMMANDER Dynamic Incubator, select the STATIC incubation method and the incubation temperature and time designated in the ASSAY PROCEDURE which follows. Use the STATIC incubation method throughout the assay. Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operations Manual may result in incorrect assay results.

COLOR DEVELOPMENT (QUANTUM II)

1. When transferring beads from wells to assay tubes, align inverted carton of tubes over their respective wells in the reaction tray. Press the tubes tightly over the wells and invert tray and tubes together so that beads fall into corresponding tubes. Blot excess water from top of tube carton.
2. Avoid strong light during Color Development.
3. Dispense acid in same tube sequence as OPD Substrate Solution.
4. Do not allow acid solution to contact metal.
NOTE: Conjugate and OPD Substrate dispensers must be rinsed with distilled or deionized water after each use. Refer to dispenser inserts for cleaning procedure.

READING (QUANTUM II)

1. Remove air bubbles prior to reading absorbance.
2. Visually inspect both substrate blanks and discard those that are contaminated (indicated by yellow-orange color). If both substrate blanks are contaminated, the run must be repeated.
3. A determination of the absorbance of the substrate blank must be made. The absorbance value of the substrate blank relative to that of the water tube must be greater than or equal to -0.020 and less than or equal to 0.040 in order for the assay to be valid. In Mode 0, blank the instrument with the water tube and read the substrate blank as a sample. (Mode 0 refers to Mode 0 on the Quantum II). Check the blank absorbance for assay validity. Stop the Mode 0 assay.
4. If the substrate blank is valid, use it to blank the instrument. Read Negative and Positive Controls, then specimens. If the substrate blank is not valid, repeat steps 3 and 4 using the alternate substrate blank.
5. If there is an interruption during the reading of samples, reblank the instrument with the substrate blank using the second substrate blank tube if necessary. Continue reading specimens.

ASSAY PROCEDURE (See Preliminary Comments and Procedural Notes)

Laboratories using the COMMANDER Flexible Pipetting Center or Parallel Processing Center should follow procedures in the appropriate Operations Manual(s). When using other automated instrumentation to deliver Controls and specimens ensure the instrumentation is compatible with this assay. Follow the manufacturer's directions to achieve the appropriate volumes and dilutions required. The following Dilution of Specimen instructions should be used when pipetting manually. The following FIRST INCUBATION and SECOND INCUBATION instructions should be used for PPC and Quantum II processing. The following COLOR DEVELOPMENT, PREPARATION OF WATER TUBE, and READING instructions should be used for Quantum II processing. For PPC color development and reading instructions, refer to the PPC Operations Manual.

CAUTION: Verify that dispensing equipment delivers specified sample and/or reagent volumes and does not introduce cross contamination.

Dilution of Specimen (manual method of sample dilution)

1a. Dispense 10 µL of each Control or specimen into the bottom of an individual test tube, pre-dilution tray or equivalent.
1b. Dispense 400 µL of Specimen Diluent to each test tube, pre-dilution well or equivalent.
1c. Ensure adequate mixing by gently tapping.
1d. Transfer 200 µL of each diluted Control or specimen into appropriate well of reaction tray.

FIRST INCUBATION (QUANTUM II and PPC)

2. Carefully add one Bead to each well containing diluted Control or specimen.
3. Apply cover seal. Tap tray gently.
4. Incubate at 40 ± 2°C for 1 hour ± 5 minutes.
5. Remove and discard cover seal. Wash each bead.

SECOND INCUBATION (QUANTUM II and PPC)

6. Pipette 200 mL of diluted Conjugate into each well containing a bead.
7. Apply new cover seal. Tap tray gently.
8. Incubate at 40 ± 2°C for 30 ± 2 minutes in a Water Bath or the COMMANDER Dynamic Incubator.
9. Remove and discard cover seal. Wash each bead.

COLOR DEVELOPMENT (QUANTUM II)

10. Immediately transfer beads to properly identified assay tubes.
11. Prime dispenser immediately prior to dispensing OPD Substrate Solution.
12. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.
13. Cover and incubate at room temperature (15 to 30°C) for 30 ± 2 minutes.
14. Add 1 mL of 1*N* Sulfuric Acid to each tube. Agitate to mix.

PREPARATION OF WATER TUBE (QUANTUM II)

15. Pipette approximately 2 mL of distilled or deionized water into an empty tube.

READING (QUANTUM II)

NOTE: Reading of the assay must be done within two hours after acid addition.
16. In Mode 0, blank the instrument with the water tube. (See appropriate Operator's manual for running Mode 0.)
17. Determine the absorbance of the substrate blank. The substrate blank must be greater than or equal to -0.020 and less than or equal to 0.040. Stop the Mode 0 assay.
18. Select mode for processing ABBOTT HCV EIA 2.0.
19. Blank the instrument with the valid substrate blank.
20. Determine the absorbance of Controls and specimens.

READING RESULTS

INSTRUMENTS

Performance of ABBOTT HCV EIA 2.0 requires the use of a precision spectrophotometer (i.e., COMMANDER System or Quantum II). REFER TO THE APPROPRIATE INSTRUMENT MANUAL FOR PROPER OPERATION AND CALIBRATION.

1. Laboratories using the COMMANDER Parallel Processing Center (PPC) must use software version 8.01/8.11 or higher. Use the assay protocol -A-HCV EIA 2.0 as provided in the software without editing. When using the PPC with the COMMANDER FPC, the Assay List Number and Assay Procedure Code in the PPC assay protocol must match the Assay List Number and the Assay Procedure Code configured on the FPC.
2. Laboratories using the Quantum II must use Module A software List Number 4045-96/4045-97 or higher. Use the assay protocol -A-HCV 2.0 EIA (Mode 1.26) as provided in the software without editing.

QUALITY CONTROL PROCEDURES[32,33]

1. Substrate Blank Acceptance Criteria
   a. **Quantum II users:** An assay run is considered valid with respect to the substrate blank if the blank has an absorbance value that is greater than or equal to -0.020 and less than or equal to 0.040. The determination of assay validity due to substrate blank must be done by user.

      The substrate blank value is an indication of the integrity of the OPD Substrate Solution. If the substrate blank absorbance falls outside the acceptable range, the preparation of the substrate is in question and the alternate substrate blank may be used. If the alternate substrate blank is unacceptable, the assay is invalid, and the run must be repeated.

   b. COMMANDER users: Quality control with respect to the substrate blank is determined automatically by the COMMANDER instrument according to the procedure described in the PPC Operations Manual. If the run is invalid, technique errors in preparation of the OPD substrate solution are suspect and the run must be repeated.
2. Negative Control Calculations and Acceptance Criteria
   a. Calculation of Results - All calculations below are performed automatically when a COMMANDER PPC or Quantum II is used.

      1) Calculation of Negative Control Mean Absorbance ($\overline{NCx}$). Determine the Mean of the Negative Control Values. Quantum II automatically subtracts the blank value from each individual Negative Control value.

         Example:

         Negative Control

Sample No.	Absorbance
1	0.080
2	0.076
3	0.079
TOTAL	0.235

$$(\overline{NCx}) = \frac{\text{Total Absorbance}}{3} = \frac{0.235}{3} = 0.078$$

   b. Negative Control Acceptance Criteria

      Individual Negative Control values must meet the following criteria:

      1) Individual Negative Control Values must be less than or equal to 0.150 and greater than or equal to 0.010.
      2) Individual Negative Control Values must be within the range 0.54 to 1.46 times the Negative Control Mean.

   If one Negative Control value does not meet either of the above criteria, it must be excluded as aberrant. The Negative Control mean must then be recalculated. All remaining individual Negative Control values must meet the above criteria or the run is invalid and must be repeated.
3. Positive Control Calculations and Acceptance Criteria
   a. Calculation of Results - All calculations below are performed automatically when a COMMANDER PPC or Quantum II is used.

      1) Calculation of Positive Control Mean Absorbance ($\overline{PCx}$). Determine the Mean of the Positive Control Values.

         Example:

         Positive Control

Sample No.	Absorbance
1	1.212
2	1.260
3	1.234
TOTAL	3.706

$$(\overline{PCx}) = \frac{\text{Total Absorbance}}{3} = \frac{3.706}{3} = 1.235$$

   b. Positive Control Acceptance Criteria

      Individual Positive Control values must meet the following criteria:

      1) Individual Positive Control Values must be less than or equal to 1.999 and greater than or equal to 0.400.
      2) Individual Positive Control Values must be within the range 0.66 to 1.34 times the Positive Control Mean.

   If one Positive Control value does not meet either of the above criteria, it must be excluded as aberrant. The Positive Control mean must then be recalculated. All remaining individual Positive Control values must meet the above criteria or the run is invalid and must be repeated.
4. Assay Run Validity Criteria

   For the run to be valid, the difference between the mean absorbances of the Positive and Negative Controls (P-N) must be 0.400 or greater. If not, technique or deterioration of reagents may be suspect and the run must be repeated. If the P-N is consistently low, deterioration of reagents may be suspect.

   Calculations for Determining P-N

   Example:

   $\overline{NCx} = 0.078$

   $\overline{PCx} = 1.235$

   P-N = (1.235 - 0.078) = 1.157

RESULTS

When a COMMANDER PPC or Quantum II is used, all calculations below are performed automatically.

1. Calculation of the Cutoff Value

   The Cutoff Value is the mean absorbance of the Negative Control plus 0.25 times the mean absorbance of the Positive Control.

   Calculation of the Cutoff Value

   Cutoff Value = $\overline{NCx}$ + (0.25) $\overline{PCx}$

   Example:

   $\overline{NCx}$ = 0.078

   $\overline{PCx}$ = 1.235

   Cutoff Value = 0.078 + (0.25)(1.235)
   = 0.078 + 0.309
   = 0.387
2. Calculation of the Unknown

   The presence or absence of antibody to HCV is determined by relating the absorbance of the unknown specimen to the Cutoff Value. If the absorbance of the unknown specimen is greater than or equal to the Cutoff Value, it is considered reactive by the criteria of ABBOTT HCV EIA 2.0.

INTERPRETATION OF RESULTS

1. Specimens with absorbance values equal to or greater than 0.005 but less than the Cutoff Value are considered negative by the criteria of ABBOTT HCV EIA 2.0.
2. Specimen results having absorbance values below 0.005 must be retested using the same product and test method to verify the initial test result as technique may be suspect. If the specimen has an absorbance value less than the Cutoff when retested, the specimen may be considered negative for antibodies by the criteria of the ABBOTT HCV EIA 2.0. Further testing is not required.
3. Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive by the criteria of ABBOTT HCV EIA 2.0, but before interpretation, the original sample must be retested in duplicate using the same product and test method. If either duplicate retest is reactive, the specimen may be interpreted to be repeatedly reactive for antibodies by the criteria of ABBOTT HCV EIA 2.0.
4. Initially reactive specimens must be repeated in duplicate using the same product and test method. If both of the duplicate repeat tests are negative, the specimen is considered negative by the criteria of the ABBOTT HCV EIA 2.0.

LIMITATIONS OF THE PROCEDURE

The ABBOTT HCV EIA 2.0 PROCEDURE and the INTERPRETATION OF RESULTS must be closely followed when testing serum, plasma, or cadaveric specimens for the presence of antibody to HCV. Performance has not been established using body fluids (e.g., urine, saliva, or pleural fluid) other than serum, plasma, or cadaveric serum. Do not use heat-inactivated specimens. A test result that is negative does not exclude the possibility of exposure to or infection with HCV. Negative results in this assay in individuals with prior exposure to HCV may be due to antibody levels below the limit of detection of this assay or lack of antibody reactivity to the HCV antigens used in this assay. Specimens may contain antibodies to either vector proteins or fusion proteins associated with the HCV recombinant antigens. Vector and/or fusion protein antibody-containing specimens may demonstrate reactivity which is unrelated to HCV infection. Additional, more specific tests may be useful in defining true HCV antibody reactivity.

EXPECTED VALUES

REACTIVITY IN RANDOM DONOR POPULATIONS

A total of 15,008 serum and plasma specimens from volunteer blood donors was evaluated at five different U.S. sites. Testing occurred in a 2-4 week period using surplus random samples which met the volume requirement of the clinical protocol. Two sites evaluated 4,669 plasma specimens and had a total of 23 (0.49%) repeatedly reactive specimens by ABBOTT HCV EIA 2.0. Four sites evaluated a total of 10,339 serum specimens by ABBOTT HCV EIA 2.0 and had a total of 72 (0.70%) repeatedly reactive specimens. One site evaluated a total of 3,509 plasmapheresis donor specimens of which 196 (5.59%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table I).

REACTIVITY IN PATIENT POPULATIONS

Four different sites evaluated a total of 319 specimens from 319 individuals diagnosed with acute or chronic non-A, non-B hepatitis (NANBH). In 130 patients with acute NANBH, 70 (53.85%) specimens were repeatedly reactive. In 189 specimens with chronic NANBH, 162 (85.71%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table II).

Four sites evaluated a total of 389 specimens from individuals with hepatitis and other liver diseases. Fifty-six (14.40%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table III).

Five sites evaluated a total of 1,217 specimens from individuals at high risk for HCV infection. This category included 251 intravenous drug abusers, 340 dialysis patients, 190 hemophiliacs, 200 homosexual males, 120 individuals anti-HBc reactive, and 116 patients with hepatocellular carcinoma. There were 553 (45.44%) specimens that were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table III).

Three sites evaluated 610 specimens with potentially interfering substances and from individuals with other diseases. Sixty-six (10.82%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table III).

TABLE I
Reactivity in Low Risk Populations and in Plasmapheresis Donors

Group	Number of Sites	Number of Specimens Tested	ABBOTT HCV EIA 2.0 Initially Reactive (%)	ABBOTT HCV EIA 2.0 Repeatedly Reactive (%)
Volunteer Blood Donors				
Plasma	2	4,669	25 (0.54%)	23 (0.49%)
Serum	4	10,339	77 (0.74%)	72 (0.70%)
Total Volunteer Donors		15,008	102 (0.68%)	95 (0.63%)
Plasmapheresis Donations	1	3,509*	198 (5.64%)	196 (5.59%)

* May include multiple donations from the same donor.

TABLE II
Reactivity in Selected Populations with Non-A, Non-B Hepatitis

Group	Number of Sites	Number of Specimens	ABBOTT HCV EIA 2.0 Initially Reactive (%)	ABBOTT HCV EIA 2.0 Repeatedly Reactive (%)
Acute[a]	2	130	73 (56.15%)	70 (53.85%)
Chronic[b]	4	189	162 (85.71%)	162 (85.71%)
Total NANBH		319	235 (73.67%)	232 (72.73%)

[a] Acute NANBH included patients with ALT levels greater than 2x the upper normal limit and negative for HBsAg, Anti-HBc IgM, and Anti-HAV IgM. Alcohol, toxic, other viral or drug related etiologies were excluded.

[b] Chronic NANBH included patients with initial and follow up serum specimens (at least 6 months between blood draws) with ALT levels greater than 2x upper normal limit unless liver biopsy indicated chronic hepatitis and negative for HBsAg. Alcohol, toxic, other viral or drug related etiologies were excluded.

TABLE III
Reactivity in Selected Populations with Hepatitis/Other Liver Diseases, Potentially Interfering Substances and Individuals at High Risk

Group	Number of Specimens Tested	ABBOTT HCV EIA 2.0 Initially Reactive (%)	ABBOTT HCV EIA 2.0 Repeatedly Reactive (%)
Hepatitis and Other Liver Diseases[a]	389	63 (16.20%)	56 (14.40%)
High Risk for HCV Infection[b]	1217	573 (47.08%)	553 (45.44%)
Potentially Interfering Substances[c]	610	70 (11.48%)	66 (10.82%)

[a] Hepatitis/Other Liver Diseases include Hepatitis A, Hepatitis B, Primary Biliary Cirrhosis, Autoimmune Hepatitis, Alcoholic Liver Disease, and Drug Induced Hepatitis.

[b] Individuals at High Risk include Intravenous Drug Users, Dialysis Patients, Hemophiliacs, Homosexual Males, Individuals anti-HBc reactive, and Patients with Hepatocellular Carcinoma.

[c] Potentially Interfering Substances include specimens from patients diagnosed with Systemic Lupus Erythematosus, Rheumatoid Arthritis, Rubella, Syphilis, HIV-1, Hypergammaglobulinemia (IgG & IgM), and other diseases (not specified).

SPECIFIC PERFORMANCE CHARACTERISTICS OF SERUM AND PLASMA TESTING

PRECISION

Assay reproducibility was determined in two studies by assaying five specimens in replicates of five in four consecutive runs at a total of ten sites. Reproducibility was determined using both manual (Quantum II) and automated (COMMANDER) assay methods. A total of eight lots of material was used to calculate inter-lot Standard Deviation (S.D.) and Percent Coefficient of Variation (%CV).

The intra-assay and inter-assay S.D. and %CV were calculated (Table IV). Mean S/CO is defined as the Mean Sample Absorbance (A_{492}) divided by the calculated Cutoff Value.

TABLE IV
ABBOTT HCV EIA 2.0 Reproducibility

Study 1 - Manual

Panel Member	N	Mean S/CO	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
High	320	3.751	0.248	6.6	0.414	11.0	0.468	12.5
Medium	319	2.725	0.192	7.0	0.299	11.0	0.327	12.0
Low	320	2.306	0.206	8.9	0.282	12.2	0.307	13.3
Borderline	320	1.082	0.129	11.9	0.163	15.0	0.165	15.3
Negative	320	0.526	0.079	15.1	0.095	18.0	0.100	19.0

Control	N	Mean OD	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
Negative	191	0.094	0.009	9.4	0.015	16.0	0.023	24.0
Positive	192	1.326	0.141	10.7	0.186	14.0	0.199	15.0

Study 1 - Automated

Panel Member	N	Mean S/CO	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
High	319	3.810	0.224	5.9	0.442	11.6	0.488	12.8
Medium	320	2.817	0.177	6.3	0.359	12.8	0.408	14.5
Low	320	2.396	0.156	6.5	0.345	14.4	0.383	16.0
Borderline	320	1.122	0.082	7.3	0.147	13.1	0.173	15.4
Negative	320	0.546	0.039	7.1	0.099	18.1	0.117	21.4

Control	N	Mean OD	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
Negative	195	0.088	0.009	9.8	0.019	21.5	0.025	28.3
Positive	195	1.283	0.081	6.3	0.134	10.4	0.160	12.5

Study 2- Automated

Panel Member	N	Mean S/CO	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
High	400	2.871	0.189	6.6	0.222	7.7	0.245	8.5
Medium	400	2.464	0.170	6.9	0.201	8.2	0.224	9.1
Low	400	1.517	0.104	6.9	0.115	7.6	0.130	8.6
Borderline	400	0.973	0.075	7.7	0.083	8.5	0.092	9.4
Negative	400	0.321	0.022	6.9	0.027	8.3	0.030	9.4

Control	N	Mean OD	Intra-Assay S.D.	Intra-Assay %CV	Inter-Assay S.D.	Inter-Assay %CV	Inter-Lot S.D.	Inter-Lot %CV
Negative	240	0.104	0.010	9.8	0.014	13.5	0.018	16.9
Positive	240	1.312	0.098	7.5	0.129	9.8	0.193	14.7

SPECIFICITY

Specificity of the ABBOTT HCV EIA 2.0 was 99.83% (14,913/14,939*) in volunteer blood donors and 99.79% (3,313/3,320*) in plasmapheresis donations based on an assumed zero prevalence of HCV antibody.

*In these calculations 69 of the 95 repeatedly reactive volunteer blood donor specimens and 189 of the 196 repeatedly reactive plasma donor specimens were excluded due to confirmation by supplemental tests which included peptide assays and an HCV immunoblot assay.

SENSITIVITY

Sensitivity of the ABBOTT HCV EIA 2.0 was evaluated by two different criteria.

The ability to detect antibody to HCV was improved from 63.3% with ABBOTT HCV EIA to 72.7% with ABBOTT HCV EIA 2.0 in patients with acute and chronic NANBH (Table V).

TABLE V
Comparison in Detection of Anti-HCV in NANBH PATIENTS

Group	Number of Specimens Tested	ABBOTT HCV EIA 2.0 Repeatedly Reactive (%)	ABBOTT HCV EIA Repeatedly Reactive (%)
Acute	130	70 (53.85%)	57 (43.85%)
Chronic	189	162 (85.71%)	145 (76.72%)
Total NANBH	319	232 (72.73%)	202 (63.32%)

The ability to detect antibody to HCV was improved from 44.3% with ABBOTT HCV EIA to 59.8% with ABBOTT HCV EIA 2.0 in patients with post-transfusion NANBH. ABBOTT HCV EIA 2.0 detected anti-HCV 3-16 weeks earlier than ABBOTT HCV EIA in 10 patients.[20]

PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING

REPRODUCIBILITY

Inter-assay reproducibility of ABBOTT HCV EIA 2.0 was assessed using 20 postmortem and 20 normal donor sera. These sera specimens were spiked with human plasma reactive for anti-HCV to create low-level reactive specimens. Each of the specimens was tested once on six different days on each of three lots of ABBOTT HCV EIA 2.0 at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 4.8% to 9.1% for the low-level reactive postmortem specimens and from 3.4% to 9.4% for the low-level reactive normal donor specimens. For inter-lot reproducibility the %CV ranged from 7.3% to 13.7% for the low-level reactive postmortem specimens and from 7.3% to 13.2% for the low-level reactive normal donor specimens.

SPECIFICITY

Specificity was evaluated using 51 postmortem and 50 normal donor specimens. Each of the specimens was tested once on each of three lots of ABBOTT HCV EIA 2.0. The mean sample to cutoff (S/CO) ratio for 153 postmortem replicates (51 specimens with three reagent lots) was 0.252 and the mean S/CO ratio for 150 normal donors replicates (50 specimens with three reagent lots) was 0.245. Results are presented in Table VI.

TABLE VI
Specificity with ABBOTT HCV EIA 2.0

Population	No. of Specimens	No. of Replicates	Mean S/CO	Negative	Initially Reactive
Postmortem	51	153	0.252	153 (100.00%)	0 (0.00%)
Normal Donor	50	150	0.245	150 (100.00%)	0 (0.00%)

The ABBOTT HCV EIA 2.0 has an estimated specificity of 100.00% (binomial confidence interval[35] = [97.62%, 100.00%]) for postmortem replicates.

SENSITIVITY

Sensitivity was evaluated using 50 postmortem and 50 normal donor specimens that were pre-screened for anti-HCV and found to be negative. These specimens were spiked with human plasma reactive for anti-HCV to create low-level reactive specimens. Each of the specimens was tested once on each of three lots of ABBOTT HCV EIA 2.0. The spiked specimens were expected to be reactive and therefore were not retested in duplicate. The mean sample to cutoff (S/CO) ratio for the 150 postmortem replicates (50 specimens with three reagent lots) was 3.103 and the mean S/CO ratio for the 150 normal donor replicates (50 specimens with three reagent lots) was 2.811. The calculated difference between the postmortem specimens and the normal donor specimens was 0.292 S/CO which was determined by the F test analysis to be statistically significant. However, all replicates of the postmortem and normal donor specimens were reactive with ABBOTT HCV EIA 2.0 resulting in 100.00% reactivity. Results are presented in Table VII.

TABLE VII
Reactivity ABBOTT HCV EIA 2.0

Population	No. of Specimens	No. of Replicates	Mean S/CO	Negative	Initially Reactive
Reactive Postmortem	50	150	3.103	0 (0.00%)	150 (100.00%)
Reactive Normal Donor	50	150	2.811	0 (0.00%)	150 (100.00%)

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