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

NOTE CHANGES HIGHLIGHTED

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

69-4837/R14
NANB AND INTENDED USE
ABBOTT HCV EIA 2.0 is an in vitro Enzyme Immunoassay for the Qualitative Detection of Antibodies to Hepatitis C Virus (anti-HCV) in Human Serum, Plasma, or Cadaveric Serum.

SUMMARY AND EXPLANATION OF TEST
The technology for enzyme immunoassay (EIA) was first described in the early 1970's. 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 EIA utilizes antigen 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 stage, 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 hepatitis C virus (HCV), from the plasma of a chronic NANBH patient who was not infected with HBV. It was later determined that individuals with NANBH who did not have detectable 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.

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 infected 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. 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 protein (c100-3) which was used in an assay to identify antibodies to HCV (anti-HCV). Further, using recombinant antigens based on the nonstructural (HC-31, c100-3) and structural (HC-34) regions of the HCV genome, a series of recombinant antigens were produced.

Several lines of evidence suggest that the single-antigen assays for detection of anti-HCV antibodies do not identify all HCV infected individuals. The ABBOTT HCV EIA 2.0 has been developed to detect antibodies to hepatitis C virus (HCV), from the plasma of a chronic NANBH chimpanzee, that included the development of a recombinant nonstructural antigen (c100-3) which was used in an assay to identify antibodies to HCV (anti-HCV), to be used on human serum, plasma, or cadaveric serum.

THE RECOMBINANT HCV PROTEINS USED IN ABBOTT HCV EIA 2.0
HCV-34 A recombinant HCV protein expressed in Escherichia coli (E. coli) contains HCV amino acids #1 to 150 presumed to encode core structural region. HC-34 is a chimeric fusion protein (E. coli) contains HCV 21 amino acids #1 to 150 presumed to encode core structural region. HCV amino acids #1569-1931 from NS3/NS4 region. c100-3 is a fusion protein with 239 amino acids of E. coli  CKS, 8 linker amino acids, HCV amino acids #1192-1457, HCV amino acids #1936-1931 (from NS3 region) and 5 linker amino acids at the carboxy terminus.

Recombinant Protein Host Organism Fusion Protein
HC-31 E. coli
c100-3 E. coli
c100-3 S. cerevisiae SOD*
HC-34 E. coli
c100-3/34 E. coli CKS*
** Superoxide Dismutase
* CMP-KDO Synthetase

SUMMARY AND EXPLANATION OF TEST
In the ABBOTT HCV EIA 2.0, human serum, plasma, or cadaveric serum is tested for the presence of anti-HCV antibodies. The ABBOTT HCV EIA 2.0 test kit is manufactured and sold under contract by Chiron Corporation under shared manufacturing agreement. Hepatitis C antigens c100-3 and HC-34 are prepared under U.S. license by Chiron Corporation. c100-3, HC-31 and HC-34 is represented below. Individual blood or plasma samples from an individual (donor or patient) has been infected with HCV, may harbor antibodies to HCV. The presence of these antibodies indicates that the donor or patients (based on clinical evaluation) may be tested for anti-HCV using the ABBOTT HCV EIA 2.0 kit and consists of:

ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

REAGENTS

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ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

REAGENTS

THE RECOMBINANT HCV PROTEINS USED IN ABBOTT HCV EIA 2.0
HCV-34 A recombinant HCV protein expressed in Escherichia coli (E. coli) contains HCV 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 expressed in E. coli contains HCV amino acids #1192-1457 presumed to encode nonstructural protein 3 (NS3) and HCV amino acids #1701-3031 presumed to encode nonstructural protein 4 (NS4). HC-31 is a chimeric protein expressed with 239 amino acids of E. coli, 8 linker amino acids, HCV amino acids #1192-1457 and 5 linker amino acids at the carboxy terminus.

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 tested for the presence of anti-HCV antibodies. The ABBOTT HCV EIA 2.0 test kit is manufactured and sold under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.

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ABBOTT HCV EIA 2.0 test kit is manufactured and sold under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.
1. Do not use kit beyond the expiration date.

Handling Precautions

1. Use accurately calibrated equipment.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

3. Avoid microbial contamination of specimens, reagents and water used for washing. Use of disposable pipettes is recommended. Avoid chemical contamination of reagents and equipment.

4. Do not pipette by mouth.

1. Bead bottle MUST be stored at room temperature

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

3. Just prior to dispensing for Color Development, seal gently to obtain a homogeneous solution. Remove the baffles from dispenser tubing, and prime dispenser prior to use.

Hazardous Chemicals

Topics

ABBOTT EIA Reagent kit.

OPD Tablet and Positive Control

NOTE: 30 µL of OPD Substrate Solution is required for each specimen or Control as well as for each substrate blank. Laboratories using the COBAS® AmpliPrep Paralleling Processing Center (iPPC™) will require approximately an additional 3 mL of OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

1. Store reagent kit at 2 to 30°C. OPD Tablets and 1N 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 reagent 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. Record the preparation time and expiration time of the OPD Substrate Solution on the container.

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

6. 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 specimen or Control.

2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using nonmetallic forceps or equivalent. Return dispensers to container immediately. Allow tablets to dissolve. Do not cap or stop the Substrate 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.

8. The Negative and Positive Controls, as provided, should be dispensed and diluted in this 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.

ABBOTT HCV EIA 2.0 MEETS FDA POTENCY REQUIREMENTS.

REAGENTS

1. Do not expose OPD reagents to strong light during storage or incubation.

2. Avoid contact of the OPD Substrate Solution and 1N Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1N Sulfuric Acid to come in contact with any metal parts. Prior to use, rinse glassware used with OPD Substrate Solution thoroughly with 1N acid (sulfuric or hydrochloric) using approximately 10% of the container volume followed by three washes of distilled water at the same volume.

3. If the dispensers obstruct the flow of beads, remove dispensers prior to dispensing beads. Replace dispensers in bead bottle, and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle.

4. Use a clean, dedicated dispenser for diluted conjugate to avoid neutralization.

5. Negative Control and Specimen Diluent. Sodium Azide has been reported to explode on percussion, such as hammering. To prevent formation of lead azide, thoroughly flush drains with water after disposing of products derived from human sources or inactivated microorganisms that 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 siphon solutions from trap using a rubber or plastic hose.

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 lactobacillus disinfectant such as 55% sodium hypochlorite, or other suitable disinfectant.3,11

5. Decommission and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.30,31

The product contains Sodium Azide as a preservative in the Positive Control, Negative Control and Specimen Diluent. Sodium Azide has been reported to explode on percussion, such as hammering. To prevent formation of lead azide, thoroughly flush drains with water after disposing of OPD tablets listed in the section of this package insert. 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:

S2 Keep out of the reach of children.

S36 Wear suitable protective clothing.

S45 In case of accident or if you feel unwell, seek medical advice immediately and show this container or label.

S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S2 Keep out of the reach of children.

S36/37/39Wear suitable protective clothing, gloves and eye/face protection.

S13 Keep away from food, drink and animal feedingstuffs.

S46 If swallowed, seek medical advice immediately and show this container or label.

S46 If swallowed, seek medical advice immediately and show this container or label.

S1/2 Keep locked up and out of the reach of children.

R40/22 Harmful: possible risks of irreversible effects if swallowed.

R35 Causes severe burns.

R43/22 Harmful in case of fire: possible release of toxic or aggressive substances from the fire.

S1/2 Keep locked up and out of the reach of children.

S45 In case of accident or if you feel unwell, seek medical advice immediately and show this container or label.

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S13 Keep away from food, drink and animal feedingstuffs.

S2 Keep out of the reach of children.

SAUER, BLOOM, UNIVERSE 3.0 MEETS FDA POTENCY REQUIREMENTS.

SAUER, BLOOM, UNIVERSE 3.0 MEETS FDA POTENCY REQUIREMENTS.
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SPECIMEN COLLECTION AND PREPARATION

ABBOTT HCV EIA 2.0 assay is designed for use 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 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 cerebrospinal fluid). Testing components or reagents in these body fluids is not recommended.

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 −20°C or colder.

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.

8. Ten negative and ten weakly reactive specimens showed no qualitative performance differences subject to five freeze-thaw cycles, however, multiple freeze-thaw cycles should be avoided. If necessary, prepare additional conjugate reagent. (See Additional Reagents Available)

9. Do not mix vials of diluted conjugate. Separate Negative and Positive Controls must be run with each assay run.

10. 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 necessary, prepare additional conjugate reagent. (See Additional Reagents Available)

PROCEDURE

Materials Provided
No. 4614 ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

See REAGENTS for a complete listing.

The list of reagents required for the COMMANDER System is found in the COMMANDER Operator’s Manual. A combination of reagents 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.

• 1X Saline Acid, No. 7312 (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 ± 5%) and 1 mL (tolerance ± 10%).

• Disposable, graduated pipettor or dispenser for measuring Diluent for OPD.

• Metal free containers for the ABBOTT Substrate Solution, can be plastic ware, or acid-washed, distilled water-filled glassware.

• Pipet Washer; or device for washing beads with a vacuum source and a double trap for retaining aspirated supernatant 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 45 ± 2°C.

• COMMANDER PPC or Quantum II.

• Bead Dispenser.

• Nitrile gloves.

• Membrane Seal Puncture Tool for acid bottles.

• ABBOTT OPD (o-Phenylenediamine2 HCl) Reagent, No. 6172.

• 1 N Sulfuric Acid, No. 7212-01.

• 20% Sulfuric Acid, No. 7312-01.

ABBOTT HCV EIA 2.0 PROCEDURE

PRELIMINARY CONSIDERATIONS

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

1. Keep all Negative Controls and all Positive Controls adjacent to the specimen(s) being tested with a labeled dialysis cup in the assay run.

2. An assay run is defined as a minimum of three Negative Controls, three Positive Controls, and 20- or 60-well tray with five run positions or a maximum of three Negative Controls, three Positive Controls, and 100 specimens on 20- or 60-well trays using a full bottle of diluted conjugate (500 tests). Use one preparation of working reagents per run.

3. 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 the Control in order to avoid cross-contamination.

1. Approximately 30 minutes prior to beginning the assay procedure, remove reagent vials from the kit and bring them to room temperature (15 ± 10°C) and mix gently. Adjust COMMANDER Dynamic Incubator, water bath or equivalent to 45 ± 2°C. 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.

2. Separate Negative and Positive Controls must be run with each bottle of diluted conjugate reagent.

3. Identify the reaction tray wells for each Control and specimen.

4. After each step, visually verify the presence of solution and bead in each well.

5. Identify the reaction tray wells for each Control and specimen.

PROCEDURE

Materials Provided
No. 4614 ABBOTT HCV EIA 2.0, 100/1000/5000 Tests

See REAGENTS for a complete listing.

The list of reagents required for the COMMANDER System is found in the COMMANDER Operator’s Manual. A combination of reagents 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.

• 1X Saline Acid, No. 7312 (Most U.S. and International locations).

• Precision pipettes and pipette tips or similar equipment to deliver 10 µL,
PROCEDURAL NOTES

SAMPLE PREPARATION 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 Protocol as indicated by your instrument.

- 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.

B. Verify reagent dispenser assignment:

<table>
<thead>
<tr>
<th>Station</th>
<th>Reagent</th>
<th>Dispenser</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>ABBOTT</td>
</tr>
<tr>
<td>2</td>
<td>OPD</td>
<td>ABBOTT</td>
</tr>
<tr>
<td>3</td>
<td>OPD</td>
<td>ABBOTT</td>
</tr>
<tr>
<td>4</td>
<td>Conjugate</td>
<td>ABBOTT</td>
</tr>
<tr>
<td>5</td>
<td>Acid</td>
<td>ABBOTT</td>
</tr>
</tbody>
</table>

NOTE: Always prime dispensers to ensure 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 "blank" tray using a separate tray. Place one blanking bead into each of the five wells, A1 through E5.

2. At the conclusion of the conjugate incubation step, press the Blank key and insert the "blank" tray, followed immediately by the first assay tray.

3. At the conclusion of the OPD incubation step, insert the "blank" 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 bottles, 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 incubation method and the incubation temperature and time designated may result in incorrect assay results.

COLOR DEVELOPMENT (QUANTUM II and PPC)

1. Pipette 200 µL of diluted Control or specimen into appropriate well of reaction tray.

2. Apply new cover seal. Tap tray gently.

3. Incubate at 40 ± 2°C for 30 ± 2 minutes in a Water Bath or the COMMANDER Dynamic Incubator.

4. Remove and discard cover seal. Wash each bead.

SECOND INCUBATION (QUANTUM II and PPC)

1. Pipette 200 µL of acid into each well containing diluted Control or specimen.

2. Apply new cover seal. Tap tray gently.

3. Incubate at 40 ± 2°C for 30 ± 2 minutes in a Water Bath or the COMMANDER Dynamic Incubator.

4. Remove and discard cover seal. Wash each bead.

COLOR DEVELOPMENT (QUANTUM II)

1. Immediately transfer beads to properly identified assay tubes.

2. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

3. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

4. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

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

READ REACTION TUBES (QUANTUM II)

1. Proceed to reading specimens.

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

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 batch if the blank is invalid.

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. There is an in-process during the reading of specimens, 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.

2. Ensure adequate mixing by gently tapping.

3. Transfer 200 µL of each diluted Control or specimen into appropriate well of reaction tray.

FIRST INCUBATION (QUANTUM II and PPC)

2. Carefully add 100 µL of acid to each well containing diluted Control or specimen.

3. Apply new 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)

5. Pipette 200 µL of acid into each well containing diluted Control or specimen.

6. Apply new cover seal. Tap tray gently.

7. Incubate at 40 ± 2°C for 30 ± 2 minutes in a Water Bath or the COMMANDER Dynamic Incubator.

8. Remove and discard cover seal. Wash each bead.

COLOR DEVELOPMENT (QUANTUM II)

10. Immediately transfer beads to properly identified assay tubes.

11. Pipettes 10 µL of acid into each well of the assay tubes containing Control and/or reagent volumes and does not introduce cross contamination.

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. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

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

READ REACTION TUBES (QUANTUM II)

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

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 to stop the Mode 0 assay.

18. Select mode for processing ABBOTT HEV EIA 2.0.

19. Blank the instrument with the valid substrate blank.

20. Determine the absorbance of Controls and specimens.

Dilution of Specimen (Autoassay 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 sample Diluent to each test tube, pre-dilution well or equivalent.

2. Ensure adequate mixing by gently tapping.

3. Transfer 200 µL of each diluted Control or specimen into appropriate well of reaction tray.

FIRST INCUBATION (QUANTUM II and PPC)

2. Carefully add 100 µL of acid to each well containing diluted Control or specimen.

3. Apply new 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)

5. Pipette 200 µL of acid into each well containing diluted Control or specimen.

6. Apply new cover seal. Tap tray gently.

7. Incubate at 40 ± 2°C for 30 ± 2 minutes in a Water Bath or the COMMANDER Dynamic Incubator.

8. Remove and discard cover seal. Wash each bead.

COLOR DEVELOPMENT (QUANTUM II)

10. Immediately transfer beads to properly identified assay tubes.

11. Pipettes 10 µL of acid into each well of the assay tubes containing Control and/or reagent volumes and does not introduce cross contamination.

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. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

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

READ REACTION TUBES (QUANTUM II)

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

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 to stop the Mode 0 assay.

18. Select mode for processing ABBOTT HEV EIA 2.0.

19. Blank the instrument with the valid substrate blank.

20. Determine the absorbance of Controls and specimens.
QUALITY CONTROL PROCEDURES

1. Substrate Blank Acceptance Criteria
   a. 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 = NCx- + 0.25(PCx-)

2. Calculation of the Unknown Absorbance
   a. Calculation of the Mean Absorbance of the Unknown Sample
      Example:
      Sample No. | Absorbance 1 | Absorbance 2 | Absorbance 3
      1            | 0.150        | 0.150        | 0.150
      2            | 0.150        | 0.150        | 0.150
      3            | 0.150        | 0.150        | 0.150
      Total Absorbance = 0.450
      Mean Absorbance = 0.150

b. Calculation of the Unknown Absorbance
   Example:
   Sample No. | Absorbance 1 | Absorbance 2 | Absorbance 3
   1            | 1.234        | 1.234        | 1.234
   2            | 1.234        | 1.234        | 1.234
   3            | 1.234        | 1.234        | 1.234
   Total Absorbance = 3.702
   Mean Absorbance = 1.234

3. Calculation of the Cutoff Value
   Example:
   NCx- = 0.078
   PCx- = 1.235
   Cutoff Value = NCx- + 0.25(PCx-) = 0.078 + 0.309 = 0.387

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. Specimens with absorbance values equal to or greater than 0.005 but less than the cutoff value may be suspect.
3. Specimens having absorbance values below 0.005 must be repeated 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 repeated, the specimen is considered negative for antibodies by the criteria of the ABBOTT HCV EIA 2.0. Further testing is not required.
4. Initially reactive specimens must be repeated in duplicate using the same product and test method. If either duplicate repeat test is reactive, the specimen may be considered positive for antibodies by the criteria of the ABBOTT HCV EIA 2.0. Additional, more specific tests may be useful in defining true HCV infection.

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 not consistent with the possibility of exposure to or infection with HCV may be due to antibody levels below the limit of detection of this assay or lack of antibody reactivity in the HCV antibodies 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 containing specimens may demonstrate reactivity which is unrelated to HCV infection. Additional, more specific tests may be useful in defining true HCV infection.
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 4,569 plasma specimens and had a total of 33 (0.49%) repeatedly reactive specimens by ABBOTT HCV EIA 2.0. Four sites evaluated a total of 10,069 serum specimens by ABBOTT HCV EIA 2.0 and had a total of 113 (1.1%) repeatedly reactive specimens. Four sites evaluated a total of 3,509 plasmapheresis donor specimens of which 196 (5.64%) 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 I).

Four sites evaluated a total of 269 specimens from individuals with hepatitis and other liver diseases. Fifty-one (14.24%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table I).

Five sites evaluated a total of 1,217 specimens from individuals at high risk for HCV infection. This category included 231 intravenous drug abusers, 340 dialysis patients, 190 hemophiliacs, 200 homosexual males, 120 individuals anti-HBs reactive, and 115 patients with hepatocellular carcinoma. There were 553 (45.44%) specimens that were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table I).

Three sites evaluated 810 specimens with potentially interfering substances and from individuals with other diseases. Sixty-nine (10.82%) specimens were repeatedly reactive by ABBOTT HCV EIA 2.0 (Table I).

### Table I

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Sites</th>
<th>Number of Specimens Tested</th>
<th>Initially Reactive (%)</th>
<th>ABBOTT HCV EIA 2.0 Reactively (%)</th>
<th>Intra-Assay Inter-Assay Inter-Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteer Blood</td>
<td>5</td>
<td>249</td>
<td>0.45</td>
<td>0.019</td>
<td>0.015</td>
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<tr>
<td>Plasma</td>
<td>10</td>
<td>706</td>
<td>0.91</td>
<td>0.027</td>
<td>0.022</td>
</tr>
<tr>
<td>Serum</td>
<td>4</td>
<td>10,339</td>
<td>0.54</td>
<td>0.019</td>
<td>0.015</td>
</tr>
<tr>
<td>Total Volunteer Blood</td>
<td>15</td>
<td>19,389</td>
<td>0.86</td>
<td>0.024</td>
<td>0.019</td>
</tr>
</tbody>
</table>

### Table II

**Reactivity in Selected Populations with Non-A, Non-B Hepatitis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Sites</th>
<th>Number of Specimens Tested</th>
<th>Initially Reactive (%)</th>
<th>ABBOTT HCV EIA 2.0 Reactively (%)</th>
<th>Intra-Assay Inter-Assay Inter-Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>2</td>
<td>130</td>
<td>0.77</td>
<td>0.027</td>
<td>0.022</td>
</tr>
<tr>
<td>Chronic</td>
<td>4</td>
<td>189</td>
<td>0.97</td>
<td>0.027</td>
<td>0.022</td>
</tr>
<tr>
<td>Total NANBH</td>
<td>5</td>
<td>319</td>
<td>0.97</td>
<td>0.027</td>
<td>0.022</td>
</tr>
</tbody>
</table>

### Table III

**Hepatitis/Other Liver Diseases Include**
- Hepatitis A
- Hepatitis B
- Primary Biliary Cirrhosis
- Autoimmune Hepatitis
- Alcoholic Liver Disease
- Drug Induced Hepatitis
- Individuals at High Risk include Intravenous Drug Users, Dialysis Patients, Hemophiliacs, Homosexual Males, Individuals anti-HBs reactive, and Patients with Hepatosplenic Carcinoma.
- Potentially Interfering Substances include specimens from patients diagnosed with Systemic Lupus Erythematosus, Rheumatoid Arthritis, Rubella, Syphilis, HIV-1, Hypogammaglobulinemia (IgG & IgM), and other diseases (not specified).

### Table IV

**Sensitivity of ABBOTT HCV EIA 2.0**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Sites</th>
<th>Number of Specimens Tested</th>
<th>Initially Reactive (%)</th>
<th>ABBOTT HCV EIA 2.0 Reactively (%)</th>
<th>Intra-Assay Inter-Assay Inter-Lot</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Total NANBH</td>
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<td>319</td>
<td>0.97</td>
<td>0.027</td>
<td>0.022</td>
</tr>
</tbody>
</table>

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

**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 I) and automated (COMMANDER) assay methods. A total of eight lots of material was used to calculate intra-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/C0 is defined as the Mean Sample Absorbance (A) divided by the calculated CutOff Value.

### Table V

**Specificity of ABBOTT HCV EIA 2.0**
Specificity of the ABBOTT HCV EIA 2.0 was 99.79% (3,313/3,320) in plasmapheresis 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.

### Table VI

**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 53.2% with ABBOTT HCV EIA to 72.7% with ABBOTT HCV EIA 2.0 in patients with acute and chronic HCV (Table V).

<table>
<thead>
<tr>
<th>Group</th>
<th>Specimens Tested</th>
<th>Reactive (%)</th>
<th>Reactivity ABBOTT HCV EIA 2.0</th>
<th>Reactivity ABBOTT HCV EIA 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>130</td>
<td>70 (53.85%)</td>
<td>57 (43.80%)</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>183</td>
<td>162 (88.11%)</td>
<td>145 (78.62%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>232 (73.72%)</td>
<td>202 (64.52%)</td>
<td></td>
</tr>
</tbody>
</table>

The ability to detect antibody to HCV was improved from 44.3% with ABBOTT HCV EIA to 58.8% with ABBOTT HCV EIA 2.0 in patients with post-transfusion NANBH. ABBOTT HCV EIA 2.0 detected HCV 3-16 weeks earlier than ABBOTT HCV EIA in 10 patients.

### PERFORMANCE CHARACTERISTICS OF CADASIVE SERESTIX

#### 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.

#### 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 at one site. The mean sample to cutoff (S/CO) ratio for 153 normal replicates (51 specimens with three reagent lots) was 0.245. Results are presented in Table VI.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>Mean S/CO</th>
<th>Reactive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Donor</td>
<td>50</td>
<td>0.886</td>
<td>100 (80.00%)</td>
</tr>
<tr>
<td>Postmortem</td>
<td>153</td>
<td>0.245</td>
<td>100 (100.00%)</td>
</tr>
</tbody>
</table>

The ABBOTT HCV EIA 2.0 has an estimated specificity of 100.0% (binomial confidence interval: 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 mean sample to cutoff (S/CO) ratio for 153 normal replicates (50 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 0.252. Results are presented in Table VII.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>Mean S/CO</th>
<th>Reactive %</th>
</tr>
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### BIBLIOGRAPHY
