Hepatitis C Virus Encoded Antigens (Recombinant c100-3, HCr43, NS5)

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

- **LOT** Lot Number
- **REF** List Number
- **IVD** In Vitro Diagnostic Medical Device
- **Caution** Consult instructions for use
- **Store at 2-8°C**
- **Store at 15-30°C**
- **Expiration Date**
- **Authorized Representative in the European Community**
- **Manufacturer**
- **Activator Line Treatment**
- **Assay Kit Card**
- **Calibrators**
- **Contains Sodium Azide. Contact with acids liberates very toxic gas.**
- **DANGER: REPRODUCTIVE HAZARD**
- **Distributed by**
- **GTIN** Global Trade Item Number
- **Line Cleaner**
- **Master Lot**
- **Pipette Tips**
- **Prime/Purge Accessories**
- **Produced for Abbott by**
- **Product of USA**
- **Purge Concentrate**
- **Reaction Trays**
- **Reagent Components**
- **Run Control Adapters**
- **Sample Cups**
- **Specimen Diluent**
- **Warning: Causes serious eye irritation**
- **Warning: May cause an allergic reaction**
- **Warning: Severe Irritant**

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

U.S. License No. 43
NAME AND INTENDED USE

The ABBOTT PRISM HCV assay is an in vitro chemiluminescent immunoassay (CHLIA) for the qualitative detection of antibodies to hepatitis C virus (anti-HCV) in human serum and plasma specimens. The ABBOTT PRISM HCV CHLIA is intended to screen individual human donors, including voluntary donors of whole blood and blood components, and other living donors for the presence of anti-HCV. It is also intended for use in screening blood and plasma specimens to screen organ donors when specimens are obtained while the donor’s heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

SUMMARY AND EXPLANATION OF THE TEST

HCV is a bloodborne virus closely associated with blood transfusion.1,2 Serological studies to detect the antibodies to recombinant antigens of HCV have established HCV as the cause of most bloodborne,3-8 as well as community acquired,9 non-A, non-B hepatitis (NANBH). Thus, the presence of anti-HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and may be capable of transmitting HCV infection.10 However, as with all immunoassays, the ABBOTT PRISM HCV assay may yield non-specific reactivity due to other causes. Although the majority of infected individuals may be asymptomatic, complications of HCV infection may include chronic hepatitis, cirrhosis, and increased risk of hepatocellular carcinoma.11-14 The implementation of screening of blood and plasma for anti-HCV has led to a marked decline in the risk of transfusion-transmitted hepatitis.15-16

The ABBOTT PRISM HCV assay is designed to detect antibodies to recombinant antigens covering the Core, NS3, NS4, and NS5 regions of the HCV genome. The relationship between the recombinant proteins used for the test and the putative structural and nonstructural proteins of the HCV genome is depicted in the diagram below. Serological studies of HCV infection indicate that antibodies may recognize any or all of the regions of the HCV genome represented on the ABBOTT PRISM HCV solid phase, thereby improving the sensitivity of the anti-HCV detection.17

The HCV43 protein, expressed in Escherichia coli (E. coli), is composed of two non-contiguous coding regions of the HCV polyprotein sequence. The first of the two regions represents amino acids 1192 to 1457 of the HCV nonstructural region 3 (NS3) protein sequence. This is followed by a second region corresponding to amino acids 1 to 150 of the HCV core protein sequence.

The c100-3 protein, expressed in Saccharomyces cerevisiae (S. cerevisiae) as a fusion protein with superoxide dismutase (SOD), includes amino acids 1569 to 1931 representing a portion of the NS3 and NS4 regions of the HCV polyprotein sequence. The NS5 protein, expressed in S. cerevisiae as a fusion protein with superoxide dismutase (SOD), includes amino acids 2054 to 2985 of the HCV polyprotein sequence. HCV antigens HCr43, c100-3, and NS5 are prepared under U.S. license, and on corresponding instrument tubing identifier labels. They are meant to facilitate identification and installation of reagent bottles within the ABBOTT PRISM System ambient reagent bay and refrigerator.

ABBOTT PRISM HCV Assay Kit (REF: D1B-68)

NOTE: Do not mix or interchange reagents from different ABBOTT PRISM HCV Assay Kits.

- **MICROPARTICLES**: 1 Bottle (325 mL) Hepatitis C Virus Encoded Antigens (Recombinant c100-3, HCr43, NS5) Coated Microparticles in phosphate buffered saline. Minimum concentration: 0.2% solids. Preservative: 0.1% sodium azide. (Symbol: ⬤)
- **CONJUGATE**: 1 Bottle (332 mL) Anti-Biotin (Mouse Monoclonal):Acridinium Conjugate (Biotinylated F(ab)2, Fragment (Goat) Anti-Human IgG (Gamma) in phosphate buffer, bovine serum albumin, and Triton X-100. Minimum concentration: 0.041µg/mL. Preservative: 0.1% sodium azide. (Symbol: ▲)
- **DILUTER**: 3 Bottles (10.4 mL each) Negative Calibrator (Human). Recalified plasma. Preservative: 0.1% sodium azide. (Symbol: NC)
- **SPECIMEN DILUENT**: 1 Bottle (328 mL) Specimen Diluent. Borate buffered saline with Tween 20, bovine serum albumin, calf serum, and Triton X-100. Preservative: 0.1% sodium azide. (Symbol: X)

Other Reagents Required

ABBOTT PRISM HCV Wash Kit (REF: D1B-58)

- **TRANSFER WASH**: 1 Bottle (3360 mL) Transfer Wash. Borate buffered saline with Tween 20, Preservative: 0.1% sodium azide. (Symbol: ☆)
- **CONJUGATE WASH**: 1 Bottle (1734 mL) Conjugate Wash. MES (2-(N-morpholino) ethanesulfonic acid) buffered saline. Preservative: 0.1% ProClon 300. (Symbol: ★)

ABBOTT PRISM Activator Concentrate (REF: 1A75-02 or 3L27-02)

- **ACTIVATOR CONCENTRATE**: 4 Bottles (900 mL each) Activator Concentrate. 0.4% hydrogen peroxide/0.06% diethylaminemipentacetic acid. ABBOTT PRISM Activator Diluent (REF: 1A75-01 or 3L27-01)

ABBOTT PRISM Positive Run Control Kit (REF: 3E60-10)

Or

ABBOTT PRISM Positive Run Control Kit (REF: 3E60-11)

NOTE: Each batch MUST end in a release control (ABBOTT PRISM Positive Control). The ABBOTT PRISM Positive Control (included in Kit REF: 3E60-10 or 3E60-11) must be used as the release control which has been configured to validate the system functionality and release sample results. Refer to the ABBOTT PRISM Run Control Kit package insert or the ABBOTT PRISM Positive Run Control Kit package insert for detailed handling and use instructions.
WARNINGS AND PRECAUTIONS

I. IV
II. For In Vitro Diagnostic Use

The performance characteristics of this product have not been established for the laboratory diagnosis of HCV infection.

The ABBOTT PRISM HCV assay meets FDA potency requirements.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product contains human sourced and/or potentially infectious components. Refer to the REAGENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced materials must be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.

Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not smoke, eat, drink, apply cosmetics, or handle contact lenses in work areas where specimens or reagents are handled.
- Clean and disinfect all spills of specimens or reagents using an appropriate disinfectant, such as 0.1% sodium hypochlorite, or other suitable disinfectants.
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state, and federal regulations.

The human plasma used in the Negative Calibrator is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV. The human plasma used in the Positive Calibrator is reactive for anti-HCV and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag and anti-HIV-1/HIV-2.

This product contains sodium azide; for a specific listing, refer to the REAGENTS section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to the Purge Concentrate:

WARNING: Contains methyisothiazolones.

H317 May cause an allergic skin reaction.

Prevention
P264 Avoid contact with the eyes, skin and clothing.

P280 Wear protective gloves / protective clothing / eye protection.

Response
P305+P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor / physician.

P308+P313 IF exposed or concerned: Get medical advice / attention.

Storage
P405 Store locked up.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to the Conjugate:

WARNING: Contains octoxynol. Contains sodium azide.

H317 Causes serious eye irritation.

Prevention
P264 Avoid contact with the eyes, skin and clothing.

P280 Wear protective gloves / protective clothing / eye protection.

Response
P301+P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice / attention.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to the Specimen Diluent:

DANGER: Contains boric acid, sodium tetraborate, octoxynol and sodium azide.

H360 May damage fertility or the unborn child.

H318 Causes serious eye damage.

H412 Harmful to aquatic life with long lasting effects.

EUH032 Contact with acids liberates very toxic gas.

Prevention
P201 Obtain special instructions before use.

P280 Wear protective gloves / protective clothing / eye protection.

Response
P305+P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor / physician.

P308+P313 IF exposed or concerned: Get medical advice / attention.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

Handling Precautions

CAUTION: The ABBOTT PRISM HCV conjugate is neutralized by contamination with human IgG. Extreme caution must be exercised when handling all containers, tubing, and accessories which may come into contact with the conjugate. Put on clean gloves before handling the ABBOTT PRISM HCV conjugate.

Do not use kits beyond the expiration date.

Gently invert each component several times prior to loading the original container on the ABBOTT PRISM System to ensure a homogenous solution. Additional gentle inversion may be required to thoroughly resuspend microparticles. Avoid foaming.

Gently invert calibrators in the calibrator pack several times prior to each use.

Each component of the ABBOTT PRISM HCV Wash Kit should be at room temperature (15°C-30°C) and then mixed before loading onto the ABBOTT PRISM System.

Do not mix reactants or calibrators from different bottles. Do not mix or interchange reactants from different ABBOTT PRISM HCV Assay Kits.

Any lot of ABBOTT PRISM HCV Wash Kit may be used with any lot of ABBOTT PRISM HCV Assay Kit.

Any lot of ABBOTT PRISM Activator Concentrate, ABBOTT PRISM Activator Diluent, and Control from ABBOTT PRISM Run Control Kit or ABBOTT PRISM Positive Run Control Kit may be used with any lot of any ABBOTT PRISM Assay Kit.

Treat Negative and Positive Calibrators and Controls as specimens.

Avoid microbial and chemical contamination of samples, reagents and equipment. The use of disposable pipette tips is recommended for any preliminary sample transfer.

Use accurately calibrated equipment.

Do not freeze reagents.

Failure to adhere to instructions in the ABBOTT PRISM Operations Manual or package insert may result in erroneous test results.

Use caution when handling samples, reagent bottles and reagent caps to prevent cross contamination.

Preparation of Activator Solution

Activator solution must be prepared by mixing equal parts of ABBOTT PRISM Activator Concentrate and ABBOTT PRISM Activator Diluent. The activator solution expires 24 hours from preparation. The ABBOTT PRISM Activator Concentrate may be used immediately after removing from the refrigerator. The volume of activator solution required for multiple tests is calculated by the ABBOTT PRISM System software. Refer to the ABBOTT PRISM Operations Manual, Section 5, PLAN WORK LOAD, for additional information. Use clean pipettes and/or metal-free containers (such as plasticware or acid-washed and purified or equivalent water-rinsed glassware) to measure. Refer to the ABBOTT PRISM Operations Manual Glossary for the definition of purified water. Prepare the activator solution.
in the bottle provided in the ABBOTT PRISM Accessory Kit (part 6A36-60). Cover the bottle opening securely with the cap provided and invert gently five to ten times to mix. Load the activator solution on the ABBOTT PRISM System. Refer to the ABBOTT PRISM Operations Manual, Section 5, PREPARE AND LOAD ACTIVATOR SOLUTION, for additional information.

**NOTE:** The activator solution must be used within 24 hours of preparation.

### Storage Instructions
- Store the ABBOTT PRISM HCV Assay Kit, ABBOTT PRISM Run Control Kit, ABBOTT PRISM Positive Run Control Kit, and ABBOTT PRISM Activator Concentrate at 2-8°C.
- Store the ABBOTT PRISM HCV Wash Kit and ABBOTT PRISM Activator Diluent at room temperature (15-30°C).
- Store ABBOTT PRISM Pipette Tips and ABBOTT PRISM Reaction Trays in their original packaging until use.
- The activator solution must be stored at 15-30°C and used within 24 hours of preparation.

### Indications of Instability or Deterioration of Reagents
The ABBOTT PRISM System will not continue to process samples when calibrator values do not meet specifications. This may indicate either deterioration or contamination of reagents, or instrument failure. Refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

### INSTRUMENT PROCEDURE
- For the software versions that may be used to perform the assay, refer to the ABBOTT PRISM Assay / Software Version Matrix located in the Supplemental Information tab of the ABBOTT PRISM Operations Manual.
- Refer to the ABBOTT PRISM Operations Manual for a detailed description of Instrument Procedures.
- Refer to the ABBOTT PRISM Operations Manual, Section 7, for limitations associated with test management.
- Solutions required for instrument cleaning and maintenance are described in detail in the ABBOTT PRISM Operations Manual, Sections 2 and 9.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ABBOTT PRISM Operations Manual, Section 9.

### SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS
- Serum (including serum collected in serum separator tubes), plasma collected in EDTA, potassium oxalate, sodium citrate, ACD-A, ACD-B, CP2D, CPD, or CPDA-1 anticoagulants, or plasma collected from segmented tubing may be used with the ABBOTT PRISM HCV assay. Follow the manufacturer’s processing instructions for serum and plasma collection tubes.
- CAUTION: Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and in Sample Net Counts/Count (VOC).
- This assay was designed and validated for use with individual human serum and plasma specimens. This assay has not been validated for use with pooled specimens.
- Do not use cadaveric plasma specimens.
- Do not use heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination.
- When shipped, specimens must be packaged and labeled in compliance with applicable regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped at 30°C or colder for a period not to exceed 7 days. Prior to freezing, the serum or plasma should be removed from the clot or red blood cells.
- Specimens may be stored for up to 14 days at 2-8°C. If storage periods greater than 14 days are anticipated, the serum or plasma should be removed from the clot or red blood cells to avoid hemolysis. Store the serum or plasma frozen (-20°C or colder).
- For cadaveric specimens, follow general standards and/or regulations for collection, storage and handling. Cadaveric specimens may be stored frozen (-20°C or colder) or stored for up to 2 days at 2-8°C. If storage periods greater than 2 days at 2-8°C are anticipated, the serum should be removed from the clot to avoid hemolysis and stored frozen.
- Previously frozen specimens must be mixed gently and thoroughly after thawing and centrifuged according to Table II in this section.
- Twenty nonreactive and 22 low-level reactive specimens showed no qualitative performance differences when subjected to 6 freeze-thaw cycles. However, some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may give erroneous or inconsistent test results.
- Clear, non-hemolysed specimens should be used when possible. Specimens containing visible particulate matter may give erroneous or inconsistent test results.
- No qualitative performance differences were observed when 20 nonreactive and 19 low-level reactive specimens were spiked with elevated levels of bilirubin (≤ 20 mg/dL), hemoglobin (≤ 500 mg/dL), red blood cells (< 0.4% v/v), triglycerides (≤ 3000 mg/dL), or protein (≤ 12 g/dL). However, specimens that contain greater concentrations of these potentially interfering substances have not been tested. The impact of greater concentrations of these potentially interfering substances on the ABBOTT PRISM HCV assay is unknown.
- Performance has not been established using umbilical cord blood or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using the ABBOTT PRISM HCV assay.
- Specimens collected by plasmapheresis, that have not been frozen, do not require centrifugation. All other specimens (including previously frozen plasmapheresis specimens) must be centrifuged as follows:

#### Non-frozen specimens
- Non-frozen specimens (excluding non-frozen plasmapheresis specimens) must be centrifuged such that g-minutes is between 30,000 and 75,000. A refrigerated or non-refrigerated centrifuge is acceptable for use. The acceptable time and force ranges that meet this criterion are listed in Table I.

<table>
<thead>
<tr>
<th>g-minutes</th>
<th>RCF (x g)</th>
<th>Centrifugation Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>3,000</td>
<td>10</td>
</tr>
<tr>
<td>30,000</td>
<td>3,000</td>
<td>15</td>
</tr>
<tr>
<td>30,000</td>
<td>3,000</td>
<td>20</td>
</tr>
<tr>
<td>30,000</td>
<td>3,000</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table I - Centrifugation Parameters

- Convert rpm to RCF as follows: RCF = 1.12 x rpm (1000)³
- Convert RCF to rpm as follows: rpm = \( \sqrt{\frac{1.12 \times RCF}{v}} \)
- RCF - The relative centrifugal force generated during centrifugation.
- rpm - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
- Centrifugation Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
- fmax - Radius of rotor in millimeters. The radius measured is dependent on whether the rotor is a fixed angle rotor or a swinging bucket rotor.
- This value is typically provided with the rotor by the manufacturer. For the fixed angle rotor, fmax is a measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor or rotor adapter. For the swinging bucket rotor, fmax is a measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor adapter or bucket at full extension.
- NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (fmax) should be manually measured in millimeters and the RCF calculated.
- g-minutes - The unit of measure for the product of RCF (x g) and centrifugation time (minutes).
- **Previously frozen specimens** must be centrifuged such that g-minutes is between 180,000 and 300,000. A refrigerated or non-refrigerated centrifuge is acceptable for use. The acceptable time and force ranges that meet this criterion are listed in Table II.

<table>
<thead>
<tr>
<th>g-minutes</th>
<th>RCF (x g)</th>
<th>Centrifugation Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180,000</td>
<td>12,000</td>
<td>15</td>
</tr>
<tr>
<td>240,000</td>
<td>12,000</td>
<td>20</td>
</tr>
<tr>
<td>300,000</td>
<td>12,000</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table II - Centrifugation Parameters

- **ANY specimen** (excluding non-frozen plasmapheresis) not tested within 24 hours of initial centrifugation, must be recentrifuged from 30,000 to 75,000 g-minutes as defined for non-frozen specimens.
- **NOTE:** Specimens retested within 24 hours of initial centrifugation do not require recentrifugation.

**FAILURE TO FOLLOW THE SPECIFIED CENTRIFUGATION PROCEDURE MAY GIVE ERRONEOUS OR INCONSISTENT TEST RESULTS.**
Centrifuged cadaveric SERUM specimens tested with ABBOTT PRISM HCV may be filtered using the instructions indicated below. If testing includes ABBOTT PRISM HIV O Plus, then the following instructions must be performed.

NOTE: Failure to adhere to the following instructions may result in erroneous or inconsistent test results for ABBOTT PRISM HIV O Plus.

Filtration of Centrifuged Cadaveric SERUM Specimens

Purified Water-rinsed or Clean Disposable Measuring Equipment

Disinfectant

Protective Disposable Gloves

PROCEDURE

The specimen volume required to perform a single assay on the ABBOTT PRISM System varies according to the number and type of assays, and the different specimen containers. The ABBOTT PRISM HCV assay requires 50 µL sample dispense. For ABBOTT PRISM Sample Cups, the minimum specimen volume required for one ABBOTT PRISM HCV assay is 350 µL.

1. Pour a minimum of 1 mL of the centrifuged cadaveric serum into the syringe.
2. While holding the filter syringe unit over the tube, insert the plunger and slowly apply pressure to deliver the filtered cadaveric serum.
3. Securely screw the syringe to the filter.

NOTE: Do not touch the tip of the filter to avoid possible contamination.

NOTE: Additional volume may be required based on the number of ABBOTT PRISM assays performed. Refer to the Specimen Volume section of this package insert.

4. If necessary, replace the clogged filter as follows:
   a. Remove the sterile filter from the package.
   b. Carefully invert the syringe to a filter-side-up position with the syringe plunger intact to prevent sample leakage. Gently remove the clogged filter and dispose of it in a potentially infectious waste container.
   c. Securely screw the syringe to the filter.
   d. If necessary, replace the clogged filter as follows:
      a. Remove the sterile filter from the package.
      b. Carefully invert the syringe to a filter-side-up position with the syringe plunger intact to prevent sample leakage. Gently remove the clogged filter and dispose of it in a potentially infectious waste container.
      c. Securely screw the syringe to the filter.
      d. Slowly apply pressure on the plunger to deliver the filtered cadaveric serum into the tube.
      e. Repeat this step as needed to successfully complete the filtration process.

NOTE: Filtered cadaveric specimens that are not tested within 24 hours of initial centrifugation must be re centrifuged, but do not need to be refiltered.

Specimen Volume

The specimen volume required to perform a single assay on the ABBOTT PRISM System varies according to the number and type of assays, and the different specimen containers. The ABBOTT PRISM HCV assay requires 50 µL sample dispense. For ABBOTT PRISM Sample Cups, the minimum specimen volume required for one ABBOTT PRISM HCV assay is 350 µL. For either primary or aliquot tubes or additional assay volume requirements, specimen volume required for one ABBOTT PRISM HCV assay is 350 µL.

Additional Materials Available

- ABBOTT PRISM HCV ASSAY PROCEDURE
- Prep 7B36-01 ABBOTT PRISM SAMPLE CUPS
- REF 1A75-10 or 3L27-10 ABBOTT PRISM ACTIVATOR LINE TREATMENT
- REF 7A03-01 or 3L00-01 ABBOTT PRISM PRIME/PURGE ACCESSORIES
- REF 7A03-30 or 3L00-30 ABBOTT PRISM PURGE CONCENTRATE
- REF 7A03-31 ABBOTT PRISM LINE CLEANER

For Cadaveric Specimens Only

- REF 2P41-01 Millipore GV Filters
- 10 cc Sterile Syringes

ABBOTT PRISM HCV ASSAY PROCEDURE

Key procedures that require operator interaction for testing samples are listed below. For detailed information concerning batch time, maximum batch size, reagent handling and loading, and associated procedural steps, refer to the ABBOTT PRISM Operations Manual, Sections 2, 5, and 7.

- Enter a Plan Work Load (refer to the ABBOTT PRISM Operations Manual, Section 5).
- Replace reagents as needed (refer to the ABBOTT PRISM Operations Manual, Sections 5 and 7).

NOTE: Gently invert each component several times prior to loading on the ABBOTT PRISM System to ensure a homogenous solution. Additional gentle inversion may be required to thoroughly resuspend microparticles. Avoid foaming. Gently invert calibrators in the calibrator pack several times prior to each use. Each component of the ABBOTT PRISM HCV Wash Kit should be at room temperature (15-30°C) and then mixed before loading onto the ABBOTT PRISM System.

- Verify that all tubing label symbols match the symbols on each reagent label. (Refer to the symbol key in the REAGENTS section of this package insert, and the ambient reagent bay and refrigerator diagrams provided with the ABBOTT PRISM System).

- Verify that all tubing is securely fastened to the corresponding wash and reagent bottles.

- Inspect the waste containers. Empty and clean as defined in the ABBOTT PRISM Operations Manual, Section 9, if necessary.

- Prepare activator solution (Refer to the Preparation of Activator Solution section of this package insert) and load onto the ABBOTT PRISM System.

- Verify that an adequate number of ABBOTT PRISM Reaction Trays are in the Tray Loader.

- Verify that an adequate number of ABBOTT PRISM Pipette Tips are in the Pipette Tip Racks.

- Perform the prime procedure (Refer to the ABBOTT PRISM Operations Manual, Section 5).

- Initiate sample processing. Gently invert calibrators in the calibrator pack several times. Open the bottles in the calibrator pack and place in the calibrator rack. Load the calibrator rack and sample racks, including the run controls. (Refer to the QUALITY CONTROL PROCEDURES, Controls, Control Handling Procedure, in this section of this package insert.)

- After the calibrators have been automatically pipetted, remove the calibrator rack. Close the calibrator bottles and return them to 2-8°C storage.

- Each specimen is initially tested once, unless the operator overrides this automatic function of the ABBOTT PRISM System.

- Sample racks may be removed after the samples have been pipetted.

NOTE: No operator interaction is required for the following steps, which are automatically carried out by the ABBOTT PRISM System: reaction tray transport, calibrator/sample/release control pipetting, incubation, reagent dispense, sample reading, data reduction, run validity and result determination.

- After specimen processing is complete, perform the purge procedure (Refer to the ABBOTT PRISM Operations Manual, Section 5). Refer to the ABBOTT PRISM Operations Manual, Section 5, for a detailed description of ChLIA procedures. The ABBOTT PRISM HCV assay is a two-step ChLIA procedure.
QUALITY CONTROL PROCEDURES

Calibration
The ABBOTT PRISM HCV Negative and Positive Calibrators are automatically tested in triplicate at the beginning of each batch. The ABBOTT PRISM System will not generate results when calibrator values do not meet specifications. This may indicate either deterioration or contamination of reagent, and may be considered invalid.

Controls
1. The ABBOTT PRISM Positive Control MUST be included as the last sample in each batch as a release control. The operator is prompted to include this control as the last sample in every batch, and the ABBOTT PRISM Positive Control is then automatically tested as a single replicate. This control must meet specifications defined in the ABBOTT PRISM Run Control Kit package insert or the ABBOTT PRISM Positive Control Kit package insert in order to validate the system functionality and release sample results. If this control does not meet specifications defined in the ABBOTT PRISM Run Control Kit package insert or the ABBOTT PRISM Positive Run Control Kit package insert, refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

2. Additional controls may be run at the operator’s discretion (refer to the ABBOTT PRISM Operations Manual, Section 3). Validate controls: Additional controls may be run anywhere within a batch as an invalid control. Specifications may be assigned to invalidating controls. If an invalid control fails to meet assigned specifications, sample processing is shutdown and no sample results are calculated or provided by the instrument. When an invalid control meets assigned specifications, sample processing continues and a valid release control (ABBOTT PRISM Positive Control) result is required to release data. Non-validating controls: Additional controls may be run anywhere within a batch as a non-validating control. Specifications may be assigned to non-validating controls. A valid release control (ABBOTT PRISM Positive Control) result is required to release data. If the user-assigned specifications for the non-validating control(s) are not met and the release control specifications are met, there will be no effect on sample processing. In this case, reactive sample results must not be considered invalid.

3. Control Handling Procedure
   a. Place run control adapters into the sample rack. The adapters can be placed in any rack position except 1, 2, 27 or 28.
   b. Place each run control bottle into an adapter in the sample rack such that when the bottle flip-top cap is opened, it can be snapped into an open position within the adapter.
   c. As mentioned above, place an ABBOTT PRISM Positive Control after the last sample tested in the batch. The controls can be placed in any rack position except 1, 2, 27, or 28.

Refer to the ABBOTT PRISM Operations Manual, Section 3, for additional information on calibrators, assay controls and run controls.

ASSAY PARAMETER SPECIFICATIONS

The ABBOTT PRISM HCV assay parameter specifications have been factory set. These parameters cannot be printed, displayed, or edited.

RESULTS

Calculation of Cutoff and S/CO Values
The ABBOTT PRISM System calculates the ABBOTT PRISM HCV assay cutoff value using the following formula:

\[
\text{Cutoff Value} = \frac{\text{Mean Negative Calibrator (NC) Net Counts} + (0.55 \times \text{Mean Positive Calibrator (PC) Net Counts})}{2.500 + (0.55 \times 30,000)} \times 19,000
\]

Example:

- Mean NC Net Counts = 2,500
- Mean PC Net Counts = 30,000
- The ABBOTT PRISM System calculates the ABBOTT PRISM HCV assay S/CO for each sample and control using the following formula:

\[
\text{S/CO} = \frac{\text{Sample Net Counts} \times \text{Cutoff Value}}{19,000}
\]

Example:

- Sample Net Counts = 32,000
  - Cutoff Value = 19,000
  - S/CO = 168

Interpretation of Results

- In the ABBOTT PRISM HCV assay, specimens with Net Counts less than the cutoff value are nonreactive and need not be tested further. Nonreactive specimens are considered negative for anti-HCV by the criteria of ABBOTT PRISM HCV.
- Specimens with Net Counts greater than or equal to the cutoff value are considered initially reactive by the criteria of ABBOTT PRISM HCV. All specimens (excluding non-frozen plasmapheresis specimens) that are reactive on initial testing must be centrifuged prior to retesting according to the table in the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert. Initially reactive specimens must be retested in duplicate using the ABBOTT PRISM HCV Assay Kit. Initially reactive specimens found within 24 hours of initial centrifugation do not require recentrifugation.
- If the sample Net Counts for both retests are less than the cutoff value, the specimen is nonreactive. Nonreactive specimens are considered negative for anti-HCV by the criteria of ABBOTT PRISM HCV.
- If the sample Net Counts for either duplicate retest are greater than or equal to the cutoff value, the specimen is considered repeatedly reactive. Repeatedly reactive results indicate the presence of anti-HCV by the criteria of ABBOTT PRISM HCV.
- Follow appropriate FDA recommendations and regulations for specimens found to be repeatedly reactive. Customers outside the U.S. must refer to their country’s government recommendations and regulations for specimens found to be repeatedly reactive.
- Samples which are repeatedly reactive may be referred for medical evaluation and additional testing.
- Additional controls may be run anywhere within a batch as a non-validating control. Specifications may be assigned to non-validating controls. A valid release control (ABBOTT PRISM Positive Control) result is required to release data. If the user-assigned specifications for the non-validating control(s) are not met and the release control specifications are met, there will be no effect on sample processing. In this case, reactive sample results must not be considered invalid.

System Errors

For a description of the error codes that appear on ABBOTT PRISM System reports, refer to the ABBOTT PRISM Operations Manual, Section 10.

LIMITATIONS OF THE PROCEDURE

- This assay was designed and validated for use with individual human serum and plasma specimens. This assay has not been validated for use with pooled specimens.
- Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and S/CO.
- Do not use heat-inactivated specimens. False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS section of this package insert for assay performance characteristics.
- Some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results. Customers outside the U.S. must refer to their country’s government recommendations and regulations for specimens found to be repeatedly reactive.
- Previously frozen specimens must be centrifuged per the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert prior to running the assay.
- An increased occurrence of drain time errors may be observed for cadaveric specimens.
- Do not use cadaveric plasma specimens. Performance has not been established using umbilical cord blood or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using the ABBOTT PRISM HCV assay.
- Do not use specimens with obvious microbial contamination, gross hemolysis, or gross hemolysis.
- Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and S/CO.
- Do not use heat-inactivated specimens.
- Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and S/CO.
SPECIFIC PERFORMANCE CHARACTERISTICS

ASSAY REPRODUCIBILITY

Assay reproducibility was determined by testing a three-member panel consisting of two diluted specimens reactive for anti-HCV (panel members 1 and 2) and one specimen nonreactive for anti-HCV (panel member 3). Panel members were prepared in recalculated human plasma. Each panel member was tested in replicates of four in five runs over five days with each of three reagent lots at six sites. In addition, each panel member was tested in replicates of four in five runs over five days with one of the three reagent lots at four of the six sites. The Negative and Positive Controls were tested once at the beginning and end of each run on each subchannel. The Negative and Positive Calibrators were automatically tested in triplicate at the beginning of each run on each subchannel. The intra-assy and inter-assy standard deviation (SD) and percent coefficient of variation (%) were determined with a variance component analysis for a mixed model (Table III).

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number of Replicates</th>
<th>Mean S/CO</th>
<th>SD %CV</th>
<th>SD %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>439</td>
<td>3.53</td>
<td>0.249</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>437</td>
<td>1.54</td>
<td>0.120</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>440</td>
<td>0.32</td>
<td>0.011</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Negative Control: 440, 0.17, 0.017, 10.3, 0.019, 11.4

Positive Control: 440, 2.34, 0.186, 7.9, 0.195, 8.3

* Cutoff Value = Mean Negative Calibrator Net Counts + (0.55 × Mean Positive Calibrator Net Counts)

intra-assay and inter-assay variability contains intra-assay variability.

TABLE III
ABBOTT PRISM HCV Assay Reproducibility

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number of Replicates</th>
<th>Mean Net Counts</th>
<th>SD %CV</th>
<th>SD %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>340</td>
<td>329.9</td>
<td>10.4</td>
<td>636.5</td>
</tr>
<tr>
<td>2</td>
<td>405</td>
<td>40.025</td>
<td>9.6</td>
<td>3,839.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number of Replicates</th>
<th>Mean Net Counts</th>
<th>SD %CV</th>
<th>SD %CV</th>
</tr>
</thead>
</table>

Negative Control: 650, 3,299, 342.3, 10.4, 636.5, 19.3

Positive Control: 650, 40.025, 3,839.8, 9.6

TABLE IV
Reactivity of the ABBOTT PRISM HCV Assay in Whole Blood and Plasmapheresis Donors, in Specimens from Individuals with Medical Conditions Unrelated to HCV Infection and in Specimens Containing Potentially Interfering Substances

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>IR (% of Total)</th>
<th>RR (% of Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>25,595</td>
<td>78 (0.30)</td>
<td>76 (0.30)</td>
</tr>
<tr>
<td>Plasmapheresis Donors</td>
<td>3,081</td>
<td>27 (0.88)</td>
<td>27 (0.88)</td>
</tr>
<tr>
<td>Total Donors</td>
<td>28,676</td>
<td>85 (0.29)</td>
<td>83 (0.29)</td>
</tr>
</tbody>
</table>

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</tr>
<tr>
<td>Total Donors</td>
<td>28,676</td>
<td>85 (0.29)</td>
<td>83 (0.29)</td>
</tr>
</tbody>
</table>

ASSAY SENSITIVITY

A total of 25,595 fresh serum and plasma specimens from volunteer whole blood donors and plasmapheresis donors were collected and tested at six geographically distinct blood centers (Table IV). Two sites tested a total of 8,252 serum specimens with initial repeat reactive rates of 0.88% (22/8,252) and 0.25% (21/8,252), respectively. Three sites tested a total of 14,262 plasma specimens with initial and repeat reactive rates of 0.20% (22/8,252) and 0.25% (21/8,252), respectively. Three sites tested a total of 8,252 serum specimens with initial and repeat reactive rates of 0.27% (22/8,252) and 0.51% (43/8,252), respectively. Three sites tested a total of 14,262 plasma specimens with initial repeat reactive rates of 0.20% (22/8,252) and 0.25% (21/8,252), respectively. Three sites tested a total of 8,252 serum specimens with initial repeat reactive rates of 0.27% (22/8,252) and 0.51% (43/8,252), respectively. Three sites tested a total of 14,262 plasma specimens with initial repeat reactive rates of 0.20% (22/8,252) and 0.25% (21/8,252), respectively.

TABLE V
ABBOTT PRISM HCV Reactivity in Specimens from Individuals Known to be anti-HCV Positive or at Increased Risk for HCV Infection

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>Number Repeatedly Reactive</th>
<th>Number Positive by Supplemental Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presumed anti-HCV</td>
<td>400</td>
<td>400 (100.00)</td>
<td>400 (100.00)</td>
</tr>
<tr>
<td>Acute Infection</td>
<td>20</td>
<td>20 (100.00)</td>
<td>20 (100.00)</td>
</tr>
<tr>
<td>Chronic Infection</td>
<td>154</td>
<td>154 (100.00)</td>
<td>154 (100.00)</td>
</tr>
<tr>
<td>Increased Risk for HCV Infection</td>
<td>260</td>
<td>151 (58.08)</td>
<td>149 (58.08)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>Number Repeatedly Reactive</th>
<th>Number Positive by Supplemental Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presumed anti-HCV</td>
<td>400</td>
<td>400 (100.00)</td>
<td>400 (100.00)</td>
</tr>
<tr>
<td>Acute Infection</td>
<td>20</td>
<td>20 (100.00)</td>
<td>20 (100.00)</td>
</tr>
<tr>
<td>Chronic Infection</td>
<td>154</td>
<td>154 (100.00)</td>
<td>154 (100.00)</td>
</tr>
<tr>
<td>Increased Risk for HCV Infection</td>
<td>260</td>
<td>151 (58.08)</td>
<td>149 (58.08)</td>
</tr>
</tbody>
</table>

* Specimens from the presumed anti-HCV positive category were only tested once.

ASSAY SPECIFICITY

A total of 834 serum and plasma repository specimens from 400 individuals known to be positive for HCV antibodies, 20 individuals with acute HCV infection, 154 individuals with chronic HCV infection, and 260 individuals at increased risk for HCV infection were tested with the ABBOTT PRISM HCV assay. Of the 834 specimens, 725 specimens (86.93%) were repeatedly reactive, and 273 specimens (99.72%) were positive by a licensed or research immunoblot assay (Table V). Overall sensitivity was estimated in these studies to be 100.00% (723/723) with a 95% confidence interval of 99.48% to 100.00%.
PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING

Reproducibility

Inter-assay reproducibility of PRISM HCV was assessed using 11 postmortem 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 in triplicate on three different days on each of three lots of PRISM HCV at one site for a total of 297 replicates. Fifteen replicates had insufficient sample volume and were excluded from the analysis. For intra-assay reproducibility, the %CV ranged from 3.4 to 11.3 for the low level reactive specimens. For inter-assay reproducibility over all lots, the percent coefficient of variation (%CV) ranged from 5.5 to 13.2 for the low-level reactive specimens. The total reproducibility ranged from 9.7 to 17.0 for the low level reactive specimens. Note: Inter-assay reproducibility includes intra-assay and inter-assay variation. Total reproducibility includes intra-assay, inter-assay and inter-lot variations.

Specificity

Specificity was evaluated using 53 postmortem donor specimens and 55 normal donor specimens. Each of the specimens was tested once on each of three lots of PRISM HCV. The mean sample to cutoff (S/CO) ratio for the 155 nonreactive postmortem replicates (53 specimens with three reagent lots; see Table VI, footnote a) was 0.15, and the mean S/CO for 165 normal donor replicates (55 specimens with three reagent lots) was 0.10. Results are presented in Table VI.

Table VI

<table>
<thead>
<tr>
<th>Population</th>
<th>Specimens</th>
<th>Replicates</th>
<th>Mean S/CO</th>
<th>Nonreactive</th>
<th>Initial Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>53</td>
<td>155</td>
<td>0.15</td>
<td>155</td>
<td>0</td>
</tr>
<tr>
<td>Normal Donor</td>
<td>55</td>
<td>165</td>
<td>0.10</td>
<td>165</td>
<td>0</td>
</tr>
</tbody>
</table>

* No results were obtained for 1 specimen on one lot due to a reagent dispenser error and 1 specimen on three lots due to drain time errors.

Sensitivity

Sensitivity was evaluated using 53 postmortem specimens and 55 normal donor specimens that were pre-screened for anti-HCV and found to be negative. The 105 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 PRISM HCV. The mean sample to cutoff (S/CO) ratio for the 150 postmortem replicates (53 specimens with three reagent lots; see Table VII, footnote a) was 1.67, and the mean S/CO ratio for the 156 normal donor replicates (54 specimens with three reagent lots; see Table VII, footnote a) was 1.64. Results are presented in Table VII.

Table VII

<table>
<thead>
<tr>
<th>Population</th>
<th>Specimens</th>
<th>Replicates</th>
<th>Mean S/CO</th>
<th>Nonreactive</th>
<th>Initial Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>51</td>
<td>155</td>
<td>1.67</td>
<td>146</td>
<td>1</td>
</tr>
<tr>
<td>Normal Donor</td>
<td>54</td>
<td>156</td>
<td>1.64</td>
<td>156</td>
<td>0</td>
</tr>
</tbody>
</table>

* No results were obtained for 1 postmortem specimen and 2 normal donor specimens using 3 reagent lots due to drain time errors.

The PRISM HCV has an estimated sensitivity of 97.33% (146/150) (95% binomial confidence interval = [93.31%-99.27%]) in postmortem serum specimens collected up to 18.8 hours after death.

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