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

Key to symbols used

For In Vitro Diagnostic Use

Store at 2-8°C

Store at 15-30°C

Consult instructions for use

Legal Manufacturer

U.S. License No. 43

ABBOTT LABORATORIES
Diagnostic Division
Abbott Park, Illinois 60064

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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 assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HCV. It is also intended for use in testing 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. Serological studies to detect the antibodies to recombinant antigens of HCV have established HCV as the cause of most bloodborne, as well as community acquired, 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.

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. The implementation of screening of blood and plasma for anti-HCV has led to a marked decline in the risk of transfusion-transmitted hepatitis.

The ABBOTT PRISM HCV assay is designed to detect antibodies to recombinant antigens covering 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.

The HCV43 protein, expressed in Escherichia coli (E. coli), is composed of contiguous and non-contiguous coding regions of the HCV polyprotein sequence. The first of the two regions represents amino acids 1 to 150 of the HCV nonstructural region 3 (NS3) protein sequence. This is followed by a second region corresponding to amino acids 150 to 225 of the NS4 region of the HCV polyprotein sequence.

The NS5 protein, expressed in Saccharomyces cerevisiae (S. cerevisiae) as a fusion protein with superoxide dismutase (SOD), includes amino acids 2560 to 2995 of the HCV polyprotein sequence. The c100-3 protein, expressed in S. cerevisiae as a fusion protein with superoxide dismutase (SOD), includes amino acids 2054 to 2995 of the HCV polyprotein sequence. HCV antigens HCr43, c100-3, and NS5 are prepared under U.S. license, by Chiron Corporation, under a shared manufacturing license, by Chiron Corporation, under a shared manufacturing agreement.

The Recombinant HCV Proteins in ABBOTT PRISM HCV:

<table>
<thead>
<tr>
<th>HCV Protein</th>
<th>Expression System</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV43</td>
<td>E. coli</td>
</tr>
<tr>
<td>c100-3</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>NS5</td>
<td>S. cerevisiae</td>
</tr>
</tbody>
</table>

The chemiluminescent signal is generated by an alkaline hydrogen peroxide solution. The resultant photons are counted.

The amount of light emitted is proportional to the amount of anti-HCV in the sample. The presence or absence of anti-HCV in the sample is determined by comparing the number of photons collected from the sample to a cutoff value determined from a calibration performed in the same batch. If the number of photons collected from a test sample is less than the cutoff value, the sample is considered nonreactive for anti-HCV by the criteria of the ABBOTT PRISM HCV assay. These specimens need not be further tested. If the number of photons collected from a test sample is greater than or equal to the cutoff value, the sample is considered reactive for anti-HCV by the criteria of the ABBOTT PRISM HCV assay. Specimens that are initially reactive must be retested in duplicate. Follow appropriate FDA recommendations and regulations for specimens found to be repeatedly reactive. For further information regarding ChLIA technology, refer to the ABBOTT PRISM Operations Manual, Section 3.

REAGENTS

• 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: MI)
• 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®
• 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: PC)

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

1. 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: MI)
2. 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®
3. 3 Bottles (10.4 mL each) Positive Calibrator (Human). Recalibrated plasma nonreactive for HBSAg, HIV-1 Ag or HIV-1 NAT, anti-HCV, and anti-HIV-1/2HIV-2. Preservative: 0.1% sodium azide. (Symbol: NC)
4. 3 Bottles (10.4 mL each) Positive Calibrator (Human). Recalibrated, inactivated plasma reactive for anti-HCV nonreactive for HBSAg, HIV-1 Ag or HIV-1 NAT, and anti-HIV-1/2HIV-2. Minimum S/CO 1.25. Preservative: 0.1% sodium azide. (Symbol: PC)
5. 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: PC)
Other Reagents Required

ABBOTT PRISM HCV Wash Kit (No. 6E19-56)
- 1 Bottle (336 mL) Transfer Wash. Borate buffered saline with Tween** 20. Preservative: 0.1% sodium azide. (Symbol --)
- 1 Bottle (1734 mL) Conjugate Wash. MES (N-morpholino)ethanesulfonic acid buffered saline. Preservative: 5% ProClin** 300. (Symbol --)

ABBOTT PRISM Activator Concentrate (No. 1A75-02)
- 4 Bottles (900 mL each) Activator Concentrates. 0.4% hydrogen peroxide/0.06% diethyleneamine/propyleneacetic acid.

ABBOTT PRISM Activator Diluent (No. 1A75-01)
- 4 Bottles (900 mL each) Activator Diluent. 0.3 N sodium hydroxide.

ABBOTT PRISM Run Control Kit (No. 3E60-10)
Or
ABBOTT PRISM Positive Run Control Kit (No. 3E60-11)

NOTE: Each batch must and in a release control (ABBOTT PRISM Positive Control). The ABBOTT PRISM Positive Control (included in Kit No. 3E60-10 or 3E60-11) must be used as the release formula 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.

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***ProClin is a registered trademark of Rohm & Haas.

WARNINGS AND PRECAUTIONS

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.

Safety Precautions

CAUTION: This product contains human sourced and/or potentially infectious materials. Components have been selected and screened to minimize contamination. Components sourced from human blood have been tested and found to be reactive for anti-HCV by FDA licensed tests. 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 ABBOTT PRISM Line Cleaner (No. 7A03-31) containing 2% tritiatedlammonium hydroxide (TEAM) may cause mild eye irritation. If this solution comes in contact with eyes, rinse immediately with water. For additional information, refer to the ABBOTT PRISM Operations Manual, Section II.
- Some components of this product contain sodium azide. For a specific listing refer to the REAGENTS section of this package insert. Sodium azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode upon percussion, such as hammering. To prevent formation of lead or copper azide, flush drains thoroughly 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 recommends the following: (1) siphon liquid from trap using a rubber or plastic hose, (2) fill drain with 10% sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.
- The components containing sodium azide are classified per the applicable European Community (EC) Directives as: Harmful (X4) and have the appropriate Risk (R) and Safety (S) phrases.

- If swallowed, seek medical advice immediately and show this container or label.
- If swallowed, seek medical advice immediately and show this container or label.
- If inhaled, seek medical advice immediately and show this container or label.
- If inhaled, seek medical advice immediately and show this container or label.
- Show this container or label.
- Show this container or label.
- Show this container or label.
- Show this container or label.
- Show this container or label.

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 with this 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 injection may be required to thoroughly resuspend microparticles. Avoid foaming. 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.
- Do not mix reagents from different bottles. Do not mix or interchange reagents from different ABBOTT PRISM HCV Wash Kits.
- Any lot of ABBOTT PRISM HCV Wash Kit can 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 Positive and Negative 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.
- Additional safety and handling precautions and limitations for the assay kit, calibrators, specimens, controls, and other reagents are described in the ABBOTT PRISM Operations Manual, Sections 7 and 8.
Preparation of Activator Solution

Activating solution must be prepared by mixing equal parts of ABBOTT PRISM Activator Concentrate and ABBOTT PRISM Activator Diluent. The activating solution expires 24 hours from preparation. The ABBOTT PRISM Activator Concentrate may be used immediately after removing from the refrigerator. The volume of activating 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 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, GLASSWARE, for the definition of supplied water. Prepare the activating solution in the bottle provided in the ABBOTT PRISM Accessory Kit (No. 6A36-60). Cover the bottle opening securely with the cap provided and invert gently five to ten times to mix. Load the activating 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 activating 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 activating 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

- ABBOTT PRISM software version 3.12 or higher must be used to perform the assay.
- 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 5 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/Cutoff Values (SNCO).

- 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 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 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-hemolyzed 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 (\(>50\) mg/dL), red blood cells (\(>0.4%\) v/v), triglycerides (\(>3000\) mg/dL), and 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 previously frozen specimens must be mixed gently and thoroughly after thawing and centrifuged according to Table II in this section.
- 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.

Preparation of Activator Solution

<table>
<thead>
<tr>
<th>Preparation Time (minutes)</th>
<th>RCF (x g)</th>
<th>g-minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,200</td>
<td>1,200</td>
</tr>
<tr>
<td>15</td>
<td>2,000-3,800</td>
<td>30,000-68,000</td>
</tr>
<tr>
<td>20</td>
<td>1,500-3,800</td>
<td>30,000-65,000</td>
</tr>
<tr>
<td>24</td>
<td>1,300-3,800</td>
<td>30,000-70,000</td>
</tr>
</tbody>
</table>

Convert rpm to RCF as follows: 

\[ RCF = \frac{1,12 \times rpm}{\sqrt{rpm}} \]

Convert RCF to rpm as follows: 

\[ rpm = \frac{RPM \times \sqrt{RF}}{1,12} \]

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/Cutoff Values (SNCO).

- 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 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.
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>Centrifugation Time (minutes)</th>
<th>RCF (x g)</th>
<th>g-minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>19,000</td>
<td>180,000</td>
</tr>
<tr>
<td>25</td>
<td>8,900-12,000</td>
<td>160,000-240,000</td>
</tr>
<tr>
<td>25</td>
<td>7,000-12,000</td>
<td>150,000-210,000</td>
</tr>
</tbody>
</table>

ANY specimen (excluding non-frozen plasmaspheresis) not tested within 24 hours of initial centrifugation, must be re-centrifuged 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 re-centrifugation.

FAILURE TO FOLLOW THE SPECIFIED CENTRIFUGATION PROCEDURE MAY GIVE ERRONEOUS OR INCONSISTENT RESULTS.

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, refer to the ABBOTT PRISM Operations Manual, Section 5.

PROCEDURE

Materials Provided

- No. 6D18-68 ABBOTT PRISM HCV Assay Kit
- No. 6D18-58 ABBOTT PRISM HCV Wash Kit
- No. 1A75-02 ABBOTT PRISM Activator Concentrate
- No. 1A75-05 ABBOTT PRISM Activator Diluent
- No. 5A07-01 ABBOTT PRISM Reaction Trays
- No. 5A07-10 ABBOTT PRISM Pipette Tips
- No. 6A36-60 ABBOTT PRISM Accessory Kit
- No. 3E60-10 ABBOTT PRISM Run Control Kit
- No. 3E60-11 ABBOTT PRISM Positive Run Control Kit
- No. 6A36-31 ABBOTT PRISM Run Control Adapters
- Protective Disposable Gloves
- Disinfectant
- Purified Water-rinsed or Clean Disposable Measuring Equipment

Additional Materials Available

- No. 7B36-01 ABBOTT PRISM Sample Cups
- PRISM Pipette
- PRISM Reaction Tube Rack
- PRISM HCV Negative and Positive Calibrators

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.

1. Enter a Plan/Work Load (refer to the ABBOTT PRISM Operations Manual, Section 5).
2. 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. 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 the tubing labels match with 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 refrigerant 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).

1. Initiate sample processing. Open the bottle 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. Control. Control Handling Procedure, in this package insert.)
2. After the calibrators have been automatically pipetted, remove the calibrator rack. Close the calibrator bottle and return them to 2-8°C storage.
3. Each specimen is initially tested once, unless the operator overrides this automatic function of the ABBOTT PRISM System.
4. 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 3, for a detailed description of ChuA procedures. The ABBOTT PRISM HCV assay is a two-step ChuA 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 reagents, or instrument failure.

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 therefore 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 Run 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).

Invalidate controls: Additional controls may be run anywhere within a batch as an invalidate control. Specifications may be assigned to invalidating controls. If an invalidate control fails to meet assigned specifications, sample processing is shutdown and no sample results are calculated or provided by the instrument. When an invalidate 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 data validation criteria are met, run will be no effect on sample processing. In this case, reactive sample results must be considered invalid.
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 Positive Control 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:

\[
\text{Cutoff Value} = \text{Mean Negative Calibrator (NC) Net Counts} + (0.55 \times \text{Mean Positive Calibrator (PC) Net Counts})
\]

Example:

- Mean NC Net Counts = 2,500
- Mean PC Net Counts = 30,000
- Cutoff Value = 19,000

The ABBOTT PRISM System calculates the ABBOTT PRISM HCV assay S/CO for each sample and control using the following formula:

\[
\text{S/CO} = \frac{\text{Sample Net Counts} \div \text{S/CO Cutoff Value}}{1.68}
\]

Example:

- Sample Net Counts = 32,000
- Cutoff Value = 19,000
- Result = 32,000 ÷ 19,000 = 1.68

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 the ABBOTT PRISM HCV assay. All specimens (excluding non-frozen plasma/serum specimens) that are reactive on initial testing must be centrifuged prior to retesting according to the tables 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.

NOTE: Specimens retested within 24 hours of initial centrifugation do not require re-centrifugation.

- 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.
- Individuals who are repeatedly reactive may be referred for medical evaluation and additional testing.
- Although the association of infectivity of donated blood or plasma and the presence of anti-HCV is strong, it is recognized that presently available methods for anti-HCV detection are not sensitive enough to detect all potentially infectious units of blood, plasma, or possible cases of HCV infection. A nonreactive test result does not exclude infection.

Reading Results

Some S/CO values may be flagged with “<” or “>” symbols. For more information on sample reports, refer to the ABBOTT PRISM Operations Manual, Section 5: Operating Instructions, Reports. The ABBOTT PRISM System reports sample results in Net Counts and S/CO. Net Counts are used by the ABBOTT PRISM System to interpret results. The S/CO value is provided in reports to show reactivity relative to the cutoff value. In the ABBOTT PRISM HCV assay, specimens with S/CO values of less than 1.00 are considered nonreactive. Specimens with an S/CO value of greater than or equal to 1.00 are considered reactive.

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 in S/CO.
- Serum from heparinized patients may be incompletely coagulated. Erroneous or inconsistent test results may occur due to the presence of fibrin. To prevent this phenomenon, draw specimen prior to heparin therapy.
- 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.
- Previously frozen specimens must be centrifuged prior to 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 bodily 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 heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination, gross hemolysis, or gross hemolysis.

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 replicate in human plasma. Each panel member was tested in replicates of four in five runs over five days with each of three reagents 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 reagents at 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-assay and inter-assay standard deviation (SD) and percent coefficient of variation (“CV”) were determined with a variance component analysis® for a mixed model® (Table III).
One site tested a total of 3,081 specimens from individuals with medical conditions unrelated to HCV infection and able IV.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Specimens Containing Medical Conditions</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>21 specimens (100.00%) were positive by a licensed immunoblot assay</td>
<td>0.249</td>
</tr>
<tr>
<td>-2</td>
<td>Forty-seven repeatedly reactive specimens determined to be negative.</td>
<td>0.017</td>
</tr>
<tr>
<td>-3</td>
<td>Ninety-nine percent (99.89%) (25,519/25,548) with a 95% confidence interval of 99.84%</td>
<td>0.01</td>
</tr>
<tr>
<td>-4</td>
<td>Ninety-nine percent (99.72%) were positive by a licensed or research immunoblot assay</td>
<td>0.017</td>
</tr>
</tbody>
</table>

ASSAY SENSITIVITY

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 (86.53%) were repeatedly reactive, and 725 specimens (99.72%) were repeatedly reactive and positive (1).

The ability of the ABBOTT PRISM HCV assay to detect HCV antibodies was evaluated by testing 10 commercially available seroconversion panels collected from blood and plasma donors who seroconverted over the course of their donation history. The ABBOTT PRISM HCV assay detected anti-HCV 3 to 14 days after the positive event.

ASSAY SPECIFICITY

A total of 25,295 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 and repeat reactive rates of 0.27% (219,252) and 0.25% (219,252), respectively. Three sites tested a total of 14,282 plasma specimens with initial and repeat reactive rates of 0.23% (281,146) and 0.20% (281,146), respectively. One site tested a total of 3,081 plasmapheresis donors with initial and repeat reactive rates of 0.88% (273,081). Based on supplemental test results from a licensed and/or research immunoblot assay and/or research HCV RNA PCR, 47 of the 76 repeatedly reactive samples were anti-HCV positive, 12 specimens were indeterminate, and 17 specimens were anti-HCV negative. Specificity based on assumed zero prevalence of anti-HCV in blood and plasmapheresis donors was estimated in these studies to be 99.95% (25,519/25,548) with a 95% confidence interval of 99.84% to 99.92%. Forty-seven repeatedly reactive specimens determined to be negative by supplemental testing were excluded from these calculations.

One site evaluated 340 serum or plasma repository specimens collected from individuals with medical conditions unrelated to HCV infection or containing potentially interfering substances (Table R). Twenty-two of the 340 specimens (6.47%) were initially reactive, and 21 of the 340 specimens (6.18%) were repeatedly reactive. All 21 specimens (100.00%) were positive by a licensed immunoblot assay.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>Number Reactively Positive at First Testing</th>
<th>Number Reactively Positive by Supplemental Testing to PRISM</th>
<th>Risk for HCV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precedent &amp; HCV Positive</td>
<td>450</td>
<td>407 (91.48)</td>
<td>407 (91.48)</td>
<td>100.00%</td>
</tr>
<tr>
<td>Plasma</td>
<td>151</td>
<td>149 (98.68)</td>
<td>149 (98.68)</td>
<td>100.00%</td>
</tr>
<tr>
<td>Chronic Infection</td>
<td>154</td>
<td>154 (100.00)</td>
<td>154 (100.00)</td>
<td>100.00%</td>
</tr>
<tr>
<td>Total</td>
<td>858</td>
<td>755 (88.03)</td>
<td>753 (88.73)</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

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>% of Total</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood Donors</td>
<td>8,252</td>
<td>22 (0.27)</td>
<td>(0.17 - 0.40)</td>
</tr>
<tr>
<td>Serum</td>
<td>21 (0.25)</td>
<td>(0.18 - 0.39)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>29 (0.35)</td>
<td>(0.23 - 0.48)</td>
<td></td>
</tr>
<tr>
<td>Plasmapheresis Donors</td>
<td>3,081</td>
<td>27 (0.88)</td>
<td>(0.58 - 1.27)</td>
</tr>
<tr>
<td>Total Donors</td>
<td>25,295</td>
<td>76 (0.30)</td>
<td>(0.24 - 0.37)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>% of Total</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Conditions</td>
<td>340</td>
<td>22 (6.47)</td>
<td>(2.67 - 9.26)</td>
</tr>
<tr>
<td>Unrelated to HCV Infection</td>
<td>21 (6.18)</td>
<td>(2.94 - 9.42)</td>
<td></td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING

Reproducibility

Inter-assay reproducibility of PRISM HCV was assessed using 11 postmortem donor sera. Three 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 PRISMHCV 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 5.7 to 15.3 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 variability. 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 reactive lots; see Table VI, footnote a) was 0.16, and the mean S/CO for 165 normal donor replicates (55 specimens with three reactive lots) was 0.10. Results are presented in Table VI.

Table VI

Reactivity with PRISM HCV

<table>
<thead>
<tr>
<th>Population</th>
<th>S/CO 0 (Nonreactive)</th>
<th>S/CO 150 (Reactive)</th>
<th>Average S/CO</th>
<th>Reactive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Donor</td>
<td>146 (100%)</td>
<td>146 (100%)</td>
<td>(100%)</td>
<td>Reactive</td>
</tr>
<tr>
<td>Mean</td>
<td>0.10</td>
<td>0.10</td>
<td>(0.00%)</td>
<td>Reactive</td>
</tr>
</tbody>
</table>

* If results were obtained for 1 postmortem specimen using 3 reagent lots due to drain time errors.

Note: S/CO ratio is the ratio of the sample to the cut-off of 0.5 for the reactive (positive) control. The S/CO of 0.5 was chosen as the cut-off for definitiveness. A S/CO of 0.10 or greater indicates a reactive result.

Sensitivity

Sensitivity was evaluated using 51 postmortem specimens and 54 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) for the 150 postmortem replicates (51 specimens, with three reactive 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 reactive lots; see Table VII, footnote a) was 1.64. Results are presented in Table VII.

Table VII

Reactivity with PRISM HCV

<table>
<thead>
<tr>
<th>Population</th>
<th>S/CO 0 (Nonreactive)</th>
<th>S/CO 150 (Reactive)</th>
<th>Average S/CO</th>
<th>Reactive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Donor</td>
<td>146 (100%)</td>
<td>146 (100%)</td>
<td>(100%)</td>
<td>Reactive</td>
</tr>
<tr>
<td>Mean</td>
<td>1.67</td>
<td>1.64</td>
<td>(0.00%)</td>
<td>Reactive</td>
</tr>
</tbody>
</table>

* If results were obtained for 1 postmortem specimen using 3 reagent lots due to drain time errors.

Note: S/CO ratio is the ratio of the sample to the cut-off of 0.5 for the reactive (positive) control. The S/CO of 0.5 was chosen as the cut-off for definitiveness. A S/CO of 0.10 or greater indicates a reactive result.

BIBLIOGRAPHY


ABBOTT LABORATORIES

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