ABBOTT PRISM® HCV
List No. 6D18
34-4266/R5

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

ABBOTT PRISM

Customer Service
United States: 1-877-4ABBOTT

Key to symbols used

REF — List Number	LOT — Lot Number
IVD — For *In Vitro* Diagnostic Use	Expiration Date
2°C – 8°C — Store at 2-8°C	ACTIVATOR DILUENT — Activator Diluent
15°C – 30°C — Store at 15-30°C	ACTIVATOR CONCENTRATE — Activator Concentrate
Consult instructions for use	EC REP — Authorized Representative
CE ABBOTT LABORATORIES Abbott Park, IL 60064 USA — Legal Manufacturer	

U.S. License No. 43

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, Illinois 60064

List No. 6D18
Printed in U.S.A.

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 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.[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 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[17] 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.[18]

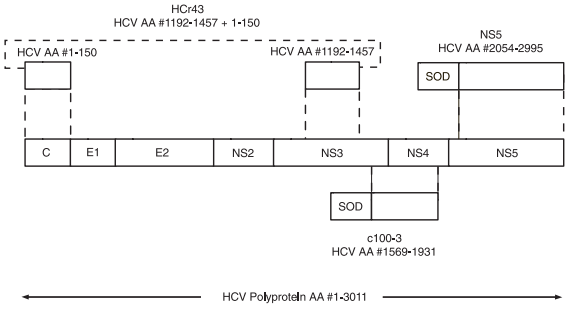
The HCr43 protein, expressed in *Escherichia coli (E. coli)*, is composed of two non-contiguous coding regions of the HCV polyprotein sequence. The first of the two regions represents amino acids 1192 to 1457 of the HCV nonstructural region 3 (NS3) protein sequence. This is followed by a second region 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 dimutase (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 dimutase (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 agreement.

The Recombinant HCV Proteins in ABBOTT PRISM HCV



BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ABBOTT PRISM HCV assay is a two-step sandwich ChLIA. The reactions occur within the ABBOTT PRISM System in the following sequence:

- Microparticles coated with HCV recombinant antigens are incubated with Specimen Diluent and sample (either plasma, serum, calibrator, or control) in the incubation well of the reaction tray. During incubation, HCV antibodies present in the sample bind to the antigen(s) on the Microparticles.
- After this first incubation is complete, the reaction mixture is transferred to the glass fiber matrix (matrix) of the reaction tray using the Transfer Wash. The Microparticles are captured by the matrix while the remaining mixture flows through to the absorbent blotter.
- The Anti-Biotin (Mouse Monoclonal):Acridinium Conjugate/ Biotinylated $F(ab')_2$ Fragment (Goat) Anti-Human IgG is added to the Microparticles on the matrix and incubated. After this second incubation, the unbound Conjugate is washed into the blotter with the Conjugate Wash.
- The chemiluminescent signal is generated by addition of 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 handled according to the table in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert and 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

NOTE: Each specific component description that follows is accompanied by a unique symbol. These symbols appear on both the component labels 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 (No. 6D18-68)

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

- 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:●)
- 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:▲)
- 3 Bottles (10.4 mL each) Negative Calibrator (Human). Recalcified plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV, and anti-HIV-1/HIV-2. Preservative: 0.1% sodium azide.(Symbol: NC)
- 3 Bottles (10.4 mL each) Positive Calibrator (Human). Recalcified, inactivated plasma reactive for anti-HCV, nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, and anti-HIV-1/HIV-2. Minimum S/CO 1.25. Preservative: 0.1% sodium azide. (Symbol: PC)
- 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 (No. 6D18-58)

- 1 Bottle (3360 mL) Transfer Wash. Borate buffered saline with Tween** 20. Preservative: 0.1% sodium azide. (Symbol: ~)
- 1 Bottle (1734 mL) Conjugate Wash. MES {2-(N-morpholino) ethanesulfonic acid} buffered saline. Preservative: 0.1% ProClin®*** 300. (Symbol:★)

ABBOTT PRISM Activator Concentrate (No. 1A75-02)

- 4 Bottles (900 mL each) Activator Concentrate. 0.4% hydrogen peroxide/0.06% diethylenetriaminepentaacetic 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** end 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 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.

*Triton is a registered trademark of Union Carbide Co., Inc.

**Tween is a registered trademark of ICI Americas.

***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 components. Some 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.[19] Biosafety Level 2[20] or other appropriate biosafety practices[21,22] 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.[23,24]
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state, and federal regulations.[25,26]

- The ABBOTT PRISM Line Cleaner (No. 7A03-31) containing 2% tetraethylammonium hydroxide (TEAH) 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 8.
- 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 (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R22 Harmful if swallowed.
R32 Contact with acids liberates very toxic gas.
S35 This material and its container must be disposed of in a safe way.
S36 Wear suitable protective clothing.
S46 If swallowed, seek medical advice immediately and show this container or label.

- The ABBOTT PRISM Activator Diluent (No. 1A75-01) contains sodium hydroxide and is classified per the applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R36 Irritating to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S46 If swallowed, seek medical advice immediately and show this container or label.

- The Conjugate Wash contains a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (3:1) which is a component of ProClin® and is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R43 May cause sensitization by skin contact.
S24 Avoid contact with skin.
S35 This material and its container must be disposed of in a safe way.
S37 Wear suitable gloves.
S46 If swallowed, seek medical advice immediately and 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 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. 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 Assay 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 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.

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

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 (No. 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

- 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 Value (S/CO).
- 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-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 (≤ 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 (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 I

Centrifugation Time (minutes)	RCF (x g)	g-minutes
10	3,000	30,000
15	2,000 - 3,000	30,000 - 45,000
20	1,500 - 3,000	30,000 - 60,000
25	1,300 - 3,000	32,500 - 75,000

Convert rpm to RCF as follows: $RCF = 1.12 \times r_{max} (rpm/1000)^2$

Convert RCF to rpm as follows: $rpm = 1000 \times \sqrt{\frac{RCF}{1.12 \times r_{max}}}$

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.

r_{max} - Radius of the 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, r_{max} is a measure of the distance from the rotor axis (center) to the bottom of the tube cavity. For the swinging bucket rotor, r_{max} is a measure of the distance from the rotor axis (center) to the bottom of the tube bucket while it is extended during rotation.

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 II

Centrifugation Time (minutes)	RCF (x g)	g-minutes
15	12,000	180,000
20	9,000 -12,000	180,000 - 240,000
25	7,200 -12,000	180,000 - 300,000

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.

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

Materials Required but not Provided

- No. 6D18-58 ABBOTT PRISM HCV Wash Kit
- No. 1A75-02 ABBOTT PRISM Activator Concentrate
- No. 1A75-01 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

  or
- 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

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. 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. 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 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 3, 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 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 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 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 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:

Cutoff Value = Mean Negative Calibrator (NC) Net Counts + (0.55 x Mean Positive Calibrator [PC] Net Counts)

Example: Mean NC Net Counts = 2,500
Mean PC Net Counts = 30,000
2,500 + (0.55 × 30,000) = 19,000
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:

S/CO = Sample Net Counts ÷ Cutoff Value

Example: Sample Net Counts = 32,000
Cutoff Value = 19,000
32,000 ÷ 19,000 = 1.68
S/CO = 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 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.

  NOTE: Specimens retested 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.
- 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 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 heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination, gross lipemia, 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 recalcified 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-assay and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were determined with a variance component analysis[27] for a mixed model[28] (Table III).

TABLE III
ABBOTT PRISM HCV Assay Reproducibility

Panel Member or Control	Number of Replicates	Mean S/CO*	Intra-Assay SD	Intra-Assay %CV	Inter-Assay[a] SD	Inter-Assay[a] %CV
1	439[b]	3.53	0.249	7.1	0.287	8.1
2	437[c]	1.54	0.120	7.8	0.140	9.1
3	440	0.12	0.011	9.2	0.013	10.8
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)

Calibrator	Number of Replicates	Mean Net Counts	Intra-Assay SD	Intra-Assay %CV	Inter-Assay SD	Inter-Assay %CV
Negative	660	3,299	342.3	10.4	636.6	19.3
Positive	659[d]	40,025	3,839.8	9.6	3,839.8	9.6

[a] Inter-assay variability contains intra-assay variability.

[b] One replicate was invalid due to instrument detection of a dispense error.

[c] Three replicates were invalid due to instrument detection of a sample to negative calibrator error, low net counts for a sample, and high dark counts for a sample.

[d] One replicate was invalid due to instrument detection of high net counts for a sample.

ASSAY SPECIFICITY

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 and repeat reactive rates of 0.27% (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% (29/14,262) and 0.20% (28/14,262), respectively. One site tested a total of 3,081 plasmapheresis donors with initial and repeat reactive rates of 0.88% (27/3,081). 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 specimens 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 99.89% (25,519/25,548) with a 95% confidence interval of 99.84% to 99.92%. Forty-seven repeatedly reactive specimens determined to be positive 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 IV). 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 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

Category	Number Tested	IR (% of Total) (95% CI)	RR (% of Total) (95% CI)
Volunteer Blood Donors			
Serum	8,252	22 (0.27) (0.17 - 0.40)	21 (0.25) (0.16 - 0.39)
Plasma	14,262	29 (0.20) (0.14 - 0.29)	28 (0.20) (0.13 - 0.28)
Plasmapheresis Donors	3,081	27 (0.88) (0.58 - 1.27)	27 (0.88) (0.58 - 1.27)
Total Donors	25,595	78 (0.30) (0.24 - 0.38)	76 (0.30) (0.23 - 0.37)
Medical Conditions Unrelated to HCV Infection and Specimens Containing Potentially Interfering Substances[a]	340	22 (6.47)	21[b] (6.18)

IR = Initial Reactive; RR = Repeat Reactive; CI = Confidence Interval

[a] Specimens from individuals with medical conditions unrelated to HCV infection and specimens containing potentially interfering substances included the following categories: anti-CMV positive (12), anti-EBV positive (12), anti-HSV positive (12), anti-HAV positive (22), HBsAg positive (12), anti-HIV-1 positive (12), anti-HIV-2 positive (5), anti-HTLV-I positive (12), anti-HTLV-II positive (12), rubella antibody positive (12), toxoplasma antibody positive (12), yeast infections (12), *E.coli* infections (5), syphilis serology positive (12), dengue antibody positive (7), anti-nuclear antibody positive (12), rheumatoid factor positive (12), influenza vaccine recipients (52), elevated IgG and elevated IgM (24), elevated triglycerides (12), elevated bilirubin (12), elevated hemoglobin (12), and non-viral liver diseases (33).

[b] The 21 repeatedly reactive specimens included the following categories: HBsAg positive (1), anti-HIV-1 positive (3), anti-HTLV-I positive (3), anti-HTLV-II positive (5), autoimmune hepatitis (3), yeast infections (5) and rheumatoid factor positive (1).

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 (86.93%) were repeatedly reactive, and 723 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 99.49% to 100.00%.

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

Category	Number Tested	Number Repeatedly Reactive (% of Total)	Number Positive By Supplemental Testing (% of RR)
Preselected anti-HCV Positive	400	400[a] (100.00)	400[a] (100.00)
Acute Infection	20	20 (100.00)	20 (100.00)
Chronic Infection	154	154 (100.00)	154 (100.00)
Increased Risk for HCV Infection[b]	260	151[c] (58.08)	149[d] (98.68)
TOTAL	834	725 (86.93)	723 (99.72)

[a] Specimens from the preselected anti-HCV positive category were only tested once.

[b] Individuals at increased risk for HCV infection included intravenous drug users (210) and hemophilia patients (50).

[c] The 151 repeatedly reactive specimens included intravenous drug users (101) and hemophilia patients (50).

[d] Of the 151 specimens, 149 were confirmed anti-HCV positive based on supplemental test results using a licensed and/or research immunoblot assay. Two of the 151 repeatedly reactive specimens were indeterminate based on the supplemental test results. All specimens in this category (260 specimens) that were repeatedly reactive by the licensed anti-HCV assay were repeatedly reactive by the PRISM assay.

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 plasmapheresis donors who seroconverted over the course of their donation history. The panels were also tested by an FDA licensed anti-HCV assay. The ABBOTT PRISM HCV assay detected anti-HCV 3 to 14 days earlier than the licensed assay in five of the 10 panels and equivalently in the other five panels.

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
Reactivity with PRISM HCV

Population	No. of Specimens	No. of Replicates	Mean S/CO	Nonreactive	Initial Reactive
Postmortem	53	155[a]	0.15	155 (100.00%)	0 (0.00%)
Normal Donor	55	165	0.10	165 (100.00%)	0 (0.00%)

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

The PRISM HCV has an estimated specificity of 100% (155/155) (95% binomial confidence interval = [97.65%-100.00%]) in postmortem serum specimens collected up to 18.8 hours after death.

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 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
Reactivity with PRISM HCV

Population	No. of Specimens	No. of Replicates	Mean S/CO	Nonreactive	Initial Reactive
Postmortem	51	150[a]	1.67	4 (2.67%)	146 (97.33%)
Normal Donor	54	156[a]	1.64	0 (0.00%)	156 (100.00%)

[a] 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 specimens collected up to 18.8 hours after death.

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