Abbott
AXSYM® SYSTEM

Anti-HCV
List No. 5C36
69-6531/R4

Anti-HCV

Customer Service
United States: 1-877-4ABBOTT
International: Call your Abbott Representative

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

CAUTION: United States Federal law restricts this device to sale and distribution by or on the order of a physician or to a clinical laboratory; and use is restricted to by or on the order of a physician.

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, Illinois 60064 USA

List No. 5C36
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NAME

AxSYM Anti-HCV

INTENDED USE

AxSYM Anti-HCV (hepatitis C virus) is a Microparticle Enzyme Immunoassay (MEIA) for the qualitative detection of anti-HCV IgG to HCV recombinant proteins in human serum or plasma (potassium EDTA, sodium EDTA, sodium heparin, lithium heparin, sodium citrate and potassium oxalate).

Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with HCV virus (state of infection or associated disease not determined) in persons with signs or symptoms of hepatitis and in persons at risk for hepatitis C infection.

WARNING:

- **Not intended for use in screening blood, plasma, or tissue donors.** The effectiveness of AxSYM Anti-HCV for use in screening blood, plasma, or tissue donors has not been established.
- Assay performance characteristics have not been established for prenatal screening or testing a pediatric population less than 10 years of age.

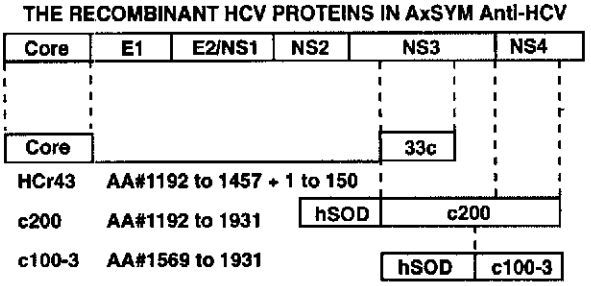
SUMMARY AND EXPLANATION OF THE TEST

AxSYM Anti-HCV is a microparticle enzyme immunoassay (MEIA), a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary analytes.[1] The bound analyte is detected by a series of antigen-antibody reactions. In the final reaction of the AxSYM MEIA, an antibody coupled to an enzyme acts upon a substrate to produce a fluorescent end-product. The fluorescence produced by the enzyme reaction is measured and, if the value is greater than or equal to the cutoff, indicates bound anti-HCV antibody.

HCV is a parenterally-transmitted virus.[2,3] Serological studies employing EIAs for detection of antibodies to recombinant antigens of HCV have established HCV as the cause of most parenterally-transmitted[4-9] as well as community-acquired[10] non-A, non-B hepatitis (NANBH) in the U.S.A. The presence of anti-HCV indicates that an individual may have been infected with HCV in the past or may have an ongoing HCV infection.[11]

AxSYM Anti-HCV has been designed to detect antibodies to putative structural and nonstructural (NS) proteins of the HCV genome. The relationship between the recombinant HCV proteins in AxSYM Anti-HCV and the putative structural and nonstructural proteins of the HCV genome is depicted in the following figure:

THE RECOMBINANT HCV PROTEINS IN AxSYM Anti-HCV



- The HCV genome encodes one large polyprotein that is processed into viral proteins. Amino acids (AA) are numbered from the amino terminus of the polyprotein. The complete HCV polyprotein contains the additional amino acids # 1932-3011 which are not represented in this figure.
- The HCr43 antigen is a protein expressed in *Escherichia coli (E. coli)* from DNA that is composed of two noncontiguous coding regions of the HCV genome. The first region represents HCV amino acids 1192 to 1457 (33c). The second region represents HCV amino acids 1 to 150 (core). The first sequence is from the NS3 coding region, and the second sequence is from the core (nucleocapsid) coding region.
- The c200 antigen is a recombinant protein expressed in *Saccharomyces cerevisiae* (yeast). It contains HCV amino acids 1192 to 1931. This sequence is from the NS3 and NS4 regions. The c200 antigen is a chimeric fusion protein, with 154 amino acids of human superoxide dismutase (hSOD).
- The c100-3 antigen is a recombinant protein expressed in *Saccharomyces cerevisiae* (yeast). The cloned HCV sequence is contained within the NS3 and NS4 regions. The c100-3 protein is a chimeric fusion protein, with 154 amino acids of hSOD, five linker amino acids, amino acids 1569 to 1931 of the HCV polyprotein, and an additional five linker amino acid chain at the carboxyl terminus.

HCV antigens HCr43, c200, and c100-3 are prepared by Chiron Corporation under a shared manufacturing agreement. The AxSYM Anti-HCV assay is manufactured under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM Anti-HCV is based on Microparticle Enzyme Immunoassay technology. The AxSYM Anti-HCV reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Anti-HCV reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).
- Sample is diluted with Specimen Diluent 1 in one RV well and then further diluted with Specimen Diluent 2 in a second RV well.
- Recombinant HCV Antigen Coated Microparticles are added to the diluted sample.

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

PROCESSING CENTER

- During the incubation of this reaction mixture, the anti-HCV in the sample binds to the Recombinant HCV Antigen Coated Microparticles, forming an antibody-antigen complex.
- A portion of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- The matrix cell is washed to remove materials not bound to the microparticles.
- Goat Anti-Human IgG:Alkaline Phosphatase Conjugate is dispensed onto the matrix cell. During the incubation of this reaction mixture, the Goat Anti-Human IgG:Alkaline Phosphatase Conjugate binds to the antibody-antigen complex.
- The matrix cell is washed to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate (MUP), is added to the Matrix Cell and the fluorescent product formed is measured by the MEIA optical assembly.

The presence or absence of anti-HCV in the sample is determined by comparing the rate of formation of fluorescent product to the cutoff rate which is calculated from a previous AxSYM Anti-HCV Index Calibration.

For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

REAGENT KIT (No. 5C36-66 includes Reagent Pack [100 tests], Reaction Vessels [100 each], and Matrix Cells [100 each] and Index Calibrator).

AxSYM Anti-HCV Reagent Pack (No. 5C36-20)

- 1 Bottle (9.9 mL) Recombinant HCV Antigen Coated Microparticles in MES buffer with protein stabilizers. Minimum Concentration: 0.05% solids. Preservative: 0.1% Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (12.1 mL) Goat Anti-Human IgG:Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.03 µg/mL. Preservative: 0.1% Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (28.0 mL) Specimen Diluent 2 containing TRIS buffer with proteins, detergents, and lysates. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents. (Reagent Bottle 3)
- 1 Bottle (50.2 mL) Specimen Diluent 1 containing Sodium Chloride in TRIS buffer. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents. (Reagent Bottle 4)

Index Calibrator

1 Bottle (4.9 mL) AxSYM Anti-HCV Index Calibrator (heat-inactivated). Recalcified human plasma, reactive for anti-HCV and nonreactive for HBsAg, HIV-1 Ag, and anti-HIV-1/HIV-2, by FDA-licensed tests. Preservative: 0.1% Sodium Azide. Dye: Green (Acid Yellow No. 23 and Acid Blue No. 9).

CONTROLS

AxSYM Anti-HCV Controls (No. 5C36-10)

2 Bottles (7.0 mL each) of AxSYM Anti-HCV Controls are prepared with recalcified human plasma. Preservative: 0.1% Sodium Azide.

The Negative Control is nonreactive for HBsAg, HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV, by FDA-licensed tests.

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The Positive Control (heat-inactivated) is reactive for anti-HCV and nonreactive for HBsAg, HIV-1 Ag, and anti-HIV-1/HIV-2, by FDA-licensed tests.

The AxSYM Anti-HCV Controls have the following ranges:

Control	Color	Anti-HCV Minimum Titer	Control Range S/CO
Negative	Natural	NA*	0.01 - 0.60
Positive	Blue†	1:2	2.50 - 7.00

* Not Applicable

† Dye: Acid Blue No. 9

OTHER REAGENTS

AxSYM Probe Cleaning Solution (No. 9A35-05)

2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (TEAH).

Solution 1 (MUP) (No. 8A47-04)

4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: Sodium Azide.

Solution 3 (Matrix Cell Wash) (No. 8A81-04)

4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

Solution 4 (Line Diluent) (No. 8A46)

1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate buffer. Preservatives: Sodium Azide and Antimicrobial Agent.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only.

CAUTION: This product contains human sourced and/or potentially infectious components. Some components sourced from human blood have been tested and found to be reactive for anti-HCV by FDA-licensed tests. Refer to REAGENTS section for details. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials 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.[12] Biosafety Level 2[13] or other appropriate biosafety practices[14,15] 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 eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.**
- **Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.[16,17]**
- **Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.[18,19]**

AxSYM Anti-HCV is susceptible to specimen carryover from AxSYM Total T3 when a high-titer anti-HCV specimen is tested by AxSYM Total T3 and is then followed by a specimen tested by AxSYM Anti-HCV. This carryover may result in a false reactive AxSYM Anti-HCV result.

SAFETY PRECAUTIONS

- The AxSYM Probe Cleaning Solution (2% TEAH) may cause mild eye irritation. If this solution comes in contact with eyes, rinse immediately with water. If irritation persists, seek medical attention.
- Some components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R 22 Harmful if swallowed.
R 32 Contact with acids liberates very toxic gas.
S 35 This material and its container must be disposed of in a safe way.
S 36 Wear suitable protective clothing.
S 46 If swallowed, seek medical advice immediately and show this container or label.

HANDLING PRECAUTIONS

- AxSYM Anti-HCV Reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. Refer to the AxSYM System Operations Manual, Section 9.
- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**
- Do not use Reagent Pack beyond the expiration date or a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different reagent packs. Do not mix reagents and index calibrators from different lots.
- Avoid microbial contamination of specimens and reagents. Use of a disposable pipette or pipette tips is recommended.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.
- Specifically for AxSYM Anti-HCV, when performing Tubing Decontamination (5% Dilution Procedure) always use sterile water where instructed to use approved water in the procedure. Refer to the AxSYM System Operations Manual, Section 9.
- Avoid chemical contamination of reagents and equipment.
- Ensure that sufficient sample volume is present. For a description of the System error codes, refer to the AxSYM System Operations Manual, Section 10.
- Inadequate adherence to package insert instructions may result in erroneous results.
- Use caution in handling patient specimens to prevent cross contamination.

Refer to the AxSYM System Operations Manual, Sections 7 and 8, for more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS

The AxSYM Anti-HCV Reagent Pack, Index Calibrator, and Controls must be stored at 2-8°C. They may be used immediately after removal from the refrigerator. The Index Calibrator and Controls should be returned to 2-8°C immediately after use.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Anti-HCV Reagent Pack may be on board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight-hour shifts. After 112 hours, the Reagent Pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendix C for further information on tracking onboard time.

Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removing it from the refrigerator. MUP may be on board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded.

The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash), and Solution 4 (Line Diluent) must be stored at 15 to 30°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Deterioration of the reagents or errors in technique may be indicated when an AxSYM Anti-HCV Positive or Negative Control value is out of the expected range. Associated test results are invalid and must be retested. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

INSTRUMENT PROCEDURE

NOTE: AxSYM Anti-HCV must only be used with AxSYM System Software Version 3.60 or higher.

ASSAY FILE INSTALLATION

The AxSYM Anti-HCV Assay File must be installed on the AxSYM System from the anti-HCV assay disk, List No. 3C37-01 or higher version, prior to performing the AxSYM Anti-HCV assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

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AxSYM ANTI-HCV ASSAY PARAMETERS

The default values for the assay parameters used for the AxSYM Anti-HCV assay are listed below. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. Press PRINT to print the assay parameters.

	Assay Parameters
1	Long Assay Name (English): Anti-HCV
6	Abbrev Assay Name (English): Anti-HCV
11	Assay Number: 842
12	Assay Version: 1
13	Calibration Version: 00
14	Assay File Revision: 103
15	Assay Enabled >ON
17	Assay Type: MEIA
18	Standard Cal Reps >2
19	Master Cal Reps: 0
20	Cal Adjust Reps: 0
21	Cal A Concentration: 1.00
22	Cal B Concentration: 0.00
23	Cal C Concentration: 0.00
24	Cal D Concentration: 0.00
25	Cal E Concentration: 0.00
26	Cal F Concentration: 0.00
27	Master Calibrator 1 Concentration: 0.00
28	Master Calibrator 2 Concentration: 0.00
29	Cal Adjust Concentration: 0.00
43	Default Dilution Protocol >UNDILUTED
44	Default Calibration Method >Index Cal
45	Selected Result Concentration Units >S/CO
46	Selected Result Decimal Places >2
62	Blank I-Max background intensity: 0.0000
63	Min Tracer-Min net intensity: 0.0000
64	Max Intercept-Max MUP intercept: 9000.0000
65	Min Intercept-Min MUP intercept: 733.0000
66	Upper limit for NRMSE for low rates: 9999.9900
67	Upper limit for NRMSE for high rates: 1.4000
68	Max Rate-Max rate used to check Min MUP Intercept: 733.0000
69	Min Rate-Rate cutoff for NRMSE and Corr. Coef.: 4.8000
70	Min correlation coefficient for low rates: 0.0000
71	Min correlation coefficient for high rates: 0.9700
72	MUP T Delay-Time delay following MUP: 3.3000
73	Low Limit-Normal/Therapeutic Range lower limit >0.00
74	High Limit-Normal/Therapeutic Range upper limit >0.00
75	Low Extreme Value >0.00
76	High Extreme Value >0.00
77	Lo Norm-% Uptake Normal Range Low >0.0000
78	High Norm-% Uptake Normal Range High >0.0000
80	Interpretation Option to use >1
84	Hold results with POS interpretation >ON
85	Hold results with NEG interpretation >OFF
86	Hold results with GRY interpretation >ON
91	Low Range Undiluted: 0.00
92	High Range Undiluted: 999999.00
96	Low Range Dil1: 0.00
97	High Range Dil1: 0.00
101	Low Range Dil2: 0.00
102	High Range Dil2: 0.00
106	Low Range Dil3: 0.00
107	High Range Dil3: 0.00
112	Max End-Point Deviation: 0.0000
113	Max Baseline Intensity: 0.0000
114	Min Baseline Intensity: 0.0000
115	Max Percent Transmission: 0.0000

NOTE: Parameters 43, 44, 45, and 80 cannot be edited.

Refer to the AxSYM System Operations Manual, Section 2, for a detailed description of Instrument Procedures.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in separator tubes) or plasma (collected in potassium EDTA, sodium EDTA, sodium heparin, lithium heparin, sodium citrate and potassium oxalate) may be used in the AxSYM Anti-HCV assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify that the correct sample type is used in the AxSYM Anti-HCV assay.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- This assay was designed and validated for use with human serum or plasma from individual specimens. Pooled specimens must not be used.
- Gravity separation is not sufficient for specimen preparation. Specimens containing red blood cells or particulate matter may give inconsistent results and must be clarified by centrifugation at a relative centrifugal force (RCF) of 10,000 x g for 10 minutes prior to testing. Specimens must be separated from clots or red blood cells by centrifugation.

  All patient specimens to be tested in primary tubes must be centrifuged to remove red blood cells or particulate matter. Each specimen that requires repeat testing, or that has been frozen and thawed, must be transferred to a centrifuge tube and centrifuged at a RCF of 10,000 x g for 10 minutes. Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top of the liquid must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen and not the lipemic material.

  NOTE: AxSYM System Software Version 3.60 and higher offers an Auto Retest/Auto Dilution feature. Due to the requirements discussed above, this feature must not be used with this assay.
- Specimens from heparinized patients may be incompletely coagulated and erroneous results could occur due to the presence of fibrin. To prevent partially coagulated specimens, draw the specimen prior to heparin therapy or into a plasma collection tube.
- Specimens may be stored on or off the clot or red blood cells for up to 14 days at 2-8°C and up to 7 days at room temperature (15 to 30°C), prior to being tested.
- Specimens which are not tested within the specified time periods must be removed from the clot or red blood cells, and stored frozen (-20°C or colder).
- Multiple freeze-thaw cycles should be avoided although no qualitative differences were observed between experimental controls and the 22 nonreactive or 22 spiked reactive specimens subjected to 6 freeze-thaw cycles. Specimens must be mixed **thoroughly** after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
- Specimens may be shipped 2-8°C, -20°C or colder, or 15 to 30°C and must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. Do not exceed the storage limitations listed above. It is recommended to ship specimens off the clot and refrigerated.
- To minimize the effects of evaporation, all samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.
- Specimens with obvious microbial contamination should be avoided.
- Do not use heat-inactivated specimens.
- Performance has not been established for use of cadaver specimens or body fluids other than human serum or plasma.
- No qualitative performance differences were observed between experimental controls and the 22 nonreactive (S/CO range: 0.18 to 0.59) or 22 spiked reactive (S/CO range: 1.88 to 4.41) specimens tested with elevated levels of bilirubin (≤ 20 mg/dL), hemoglobin (≤ 500 mg/dL), lipids (≤ 3000 mg/dL), total protein (≤ 12 g/dL), or red blood cells (≤ 0.4% v/v).
- Frozen specimens and those containing particulate matter must be clarified by centrifugation prior to testing.

SAMPLE VOLUME

The sample volume required to perform a single HCV test on the AxSYM System varies according to the type of sample container used. For sample cups, a ROUTINE test requires 150 µL and a STAT test requires 83 µL. For every additional AxSYM Anti-HCV test performed (ROUTINE or STAT) from the same sample container, an additional 33 µL of sample is required.

The sample cup minimum volumes for both STAT and ROUTINE tests are calculated by the AxSYM System. It is displayed on the Orderlist screen at

the time the test(s) is(are) ordered and printed in the Orderlist Report. When using Host Order Query, the Orderlist screen information and Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Query Option.

To obtain the recommended volume requirements for the AxSYM Anti-HCV Index Calibrator and Controls, hold the bottles **vertically**, and dispense 4 drops of Index Calibrator or 4 drops of Positive or Negative Control into each respective sample cup.

For sample volume requirements in Primary or Aliquot Tubes, and control volume requirements for multiple AxSYM Anti-HCV reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM ANTI-HCV PROCEDURE

MATERIALS REQUIRED

- No. 5C36-66 — AxSYM Anti-HCV Reagent Kit, containing:
  AxSYM Anti-HCV Reagent Pack
  AxSYM Anti-HCV Index Calibrator
  100 Reaction Vessels (RV)
  100 Matrix Cells
- No. 5C36-10 — AxSYM Anti-HCV Controls
- No. 8A47-04 — Solution 1 (MUP)
- No. 8A81-04 — Solution 3 (Matrix Cell Wash)
- No. 8A46 — Solution 4 (Line Diluent)
- No. 9A35-05 — AxSYM Probe Cleaning Solution
- No. 8A76-01 — Sample Cups

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes or pipette tips

CAUTION:

- AxSYM Anti-HCV Index Calibrator and Controls should be mixed by gentle inversion prior to use.
- When manually dispensing samples, verify that dispensing equipment does not introduce cross contamination, and that it delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.

PROCEDURE

1. **CAUTION:** The System status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, reagent packs, or RVs.
2. **NOTE:** The AxSYM System Auto Retest/Auto Dilution feature must **not** be used. See **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section.
3. Check for sufficient onboard inventory of Matrix Cells, bulk solutions, and sample segment availability.
4. Check for sufficient waste collection capacity.

   CAUTION: Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in progress. If opened, all processing will stop. All tests will be terminated and must be repeated.
5. Order the AxSYM Anti-HCV Index Calibrator, Controls, and/or patient specimens as required. Assign or modify sample segment position (S/P) for each sample, as necessary. See **QUALITY CONTROL PROCEDURES** section for calibration and control requirements.

   Index Calibration:

   Perform AxSYM Anti-HCV calibration by testing 2 replicates of the Index Calibrator. Dispense at least 4 drops of the Index Calibrator into a sample cup. Do not simultaneously calibrate more than one AxSYM Anti-HCV reagent lot.

   Controls:

   Perform quality control by testing Positive and Negative Controls. Dispense 4 drops each of the Positive and Negative Controls into individual sample cups. When more than one AxSYM Anti-HCV reagent lot is on board the AxSYM System, multiply the control volume by the number of lots.

   Patient Specimens:

   Ensure that sufficient volume is present in the sample cups or tubes. For routine tests using sample cups, the minimum sample volume required is 150 µL. For STAT tests using sample cups, the minimum sample volume required is 83 µL. For every additional AxSYM Anti-HCV test performed from the same sample container, an additional 33 µL sample will be required. For volume requirements in Primary or Aliquot Tubes, refer to the AxSYM System Operations Manual, Section 5.

   NOTE: The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum STAT sample cup volume requirements for all tests ordered. When using the Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Query Option.
6. Place sample segments containing the ordered samples into the Sample Carousel.
7. Open Reagent Bottle 4 containing the Specimen Diluent 1. Place the AxSYM Anti-HCV Reagent Pack into the Reagent Pack Carousel.
8. Ensure that RVs are present on the RV Carousel. Additional RVs may be added as needed.
9. Press RUN. All entries on the Orderlist screen are transferred to the Order Status screen for sample processing.
10. Review the results to determine whether retesting is required.
11. Close Reagent Bottle 4 and remove the samples and the AxSYM Anti-HCV Reagent Pack from the Sampling Center when testing is completed. Store at 2-8°C.

NOTE: When using the onboard reagent tracking feature, the operator must perform a reagent pack scan after removing any pack from the system in order to maintain the validity of the reagent pack stability time.

Refer to Sections 5 and 6 of the AxSYM System Operations Manual for a detailed explanation of performing assay calibration and sample testing procedures.

QUALITY CONTROL PROCEDURES

CALIBRATION

A minimum of two replicates of the AxSYM Anti-HCV Index Calibrator must be tested for an AxSYM Anti-HCV calibration. A single sample of both the Positive and Negative Controls must be tested as a means of evaluating the assay calibration. Once an AxSYM Anti-HCV calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used for an assay.
- Either of the AxSYM Anti-HCV Control values is out of its specified range.
- The MEIA Optics Verification Update has been performed.

Refer to the AxSYM System Operations Manual, Section 6 for:

- Setting up an assay calibration
- When recalibration may be necessary
- Calibration verification

The operator must verify that the AxSYM Anti-HCV Control values are within the acceptable ranges specified in this package insert (see CONTROLS section).

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The performance of the Abbott AxSYM Anti-HCV Controls has not been established with any other anti-HCV assays.

Since the AxSYM Anti-HCV Controls are human serum based (recalcified plasma) they may not adequately control the assay when plasma specimens are assayed.

Good laboratory practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of the assay procedure. Positive Control and Negative Control provided are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Quality Control requirements must be performed in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's Standard Quality Control procedures. It is recommended that the user refer to Internal Quality Control Testing: Principles and Definitions or other published guidelines for general quality control recommendations. [NCCLS. Internal Quality Control: Principles and Definitions; Approved Guideline. NCCLS document C24-A (ISBN 1-56238-112-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1991.] and 42 CFR 493.1202 (c) for guidance on appropriate QC practices.

The minimum control requirement for an AxSYM Anti-HCV assay is a single sample of each of the Positive and Negative Controls tested once every 8 hour shift for each reagent lot. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls, follow those procedures.

The AxSYM Anti-HCV Control values must be within the acceptable ranges specified in this package insert (see CONTROLS section). If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

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Deterioration of the reagents or errors in technique may be indicated when an AxSYM Anti-HCV Positive or Negative Control value is out of the expected range. If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

Fluorescence Background Acceptance Criteria

Quality control with regard to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64, Max Intercept-Max MUP Intercept, each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2, for further information on parameter files.

RESULTS

CALCULATION

The AxSYM System calculates the cutoff rate from the mean rate of two Index Calibrator replicates and stores the result. The cutoff rate (CO) is determined by multiplying the AxSYM Anti-HCV Index Calibrator mean rate by 0.12.

Cutoff Rate (CO) = 0.12 x Index Calibrator mean rate

The AxSYM Anti-HCV assay protocol calculates a result based on the ratio of the sample rate (S) to the cutoff rate (CO) for each sample and control.

$$S/CO = \frac{\textit{Sample Rate (S)}}{\textit{Cutoff Rate (CO)}}$$

FLAGS

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2.

INTERPRETATION OF RESULTS

Initial AxSYM Anti-HCV Results

Initial Result	Instrument Flag	Interpretation	Retest Procedure
≥ 1.21	REACTIVE	Reactive	No retest required
0.80 to 1.20*	GRAYZONE	Grayzone	Retest in duplicate
0.00 to 0.79	NONREACTIVE	Nonreactive	No retest required

*Specimens with an S/CO within the grayzone range should be centrifuged according to directions in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section and retested in duplicate.

Final AxSYM Anti-HCV Results

AxSYM Anti-HCV Initial Result	AxSYM Anti-HCV Retest Results	AxSYM Anti-HCV Final Result	Interpretation
Reactive	No retest required	Reactive	Presumptive evidence of antibodies to HCV; follow CDC recommendations for supplemental testing.*
	Both of the duplicate retests are reactive.	Reactive	Presumptive evidence of antibodies to HCV; follow CDC recommendations for supplemental testing.*
Grayzone	One or both of the duplicate retests are repeatedly in the grayzone or one retest is reactive and the other nonreactive.	Equivocal	Antibodies to HCV may or may not be present; another specimen should be obtained from the individual for further testing.
	Both of the duplicate retests are nonreactive.	Nonreactive	Antibodies to HCV not detected; does not exclude early acute HCV infection.
Nonreactive	No retest required.	Nonreactive	Antibodies to HCV not detected; does not exclude early acute HCV infection.

*CDC. Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-Related Chronic Disease. MMWR 1998:47 (RR-19)

Current methods for the detection of antibodies to HCV may not detect all infected individuals. A nonreactive test result does not exclude the possibility of exposure to HCV or early acute infection with HCV. Nonreactive test results in individuals with prior exposure to HCV may be due to antibody levels being below the detection limit of this assay or to lack of antibody reactivity to the antigens used in this assay.

Results obtained with the AxSYM Anti-HCV assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

The magnitude of a AxSYM Anti-HCV assay result cannot be correlated to an end point titer.

Plasma containing liquid anticoagulants may lower the signal/cutoff (S/CO) values in some anti-HCV reactive samples. High negative results obtained on samples collected with liquid anticoagulants should be interpreted accordingly. Additional testing may be required.

LIMITATIONS OF THE PROCEDURE

- Results from immunosuppressed individuals should be interpreted with caution.
- The prevalence of the analyte in different populations will affect the assay's predictive value.
- The cross-reactivity of the AxSYM Anti-HCV assay with other flaviviruses known to cause hepatic disease has not been established.
- Specimens containing antibodies to the vector or fusion proteins may demonstrate reactivity which is unrelated to HCV infection. Additionally, more specific supplemental tests should be used to determine true anti-HCV reactivity or to detect the HCV genome.
- Positive results do not discriminate between active (asymptomatic, acute, chronic) or inactive (resolved) HCV infections. The effect of the type of HCV infection or associated disease on the performance of AxSYM Anti-HCV is not known.
- This assay was designed and validated for use with human serum or plasma (EDTA, heparin, sodium citrate and potassium oxalate) from individual patient specimens. Pooled specimens must not be used.

EXPECTED VALUES

AxSYM Anti-HCV reactivity was determined in three distinct populations representing individuals at low risk for HCV infection for whom this assay is not typically indicated (Table I). Testing with a FDA-licensed version 2.0 HCV strip immunoblot assay was also performed to provide supplemental evidence for the presence of anti-HCV among specimens that were reactive by AxSYM Anti-HCV.

Table I

AxSYM Anti-HCV Reactivity in Random Hospital Patients and Volunteer Whole Blood Donors#

Population	Number of Specimens Tested	AxSYM Anti-HCV Results (% of Total)	Supplemental Assay Positive* (% of AxSYM Anti-HCV Reactive)
Random Hospital Patients	1,003	37 (3.7%) Reactive 4 (0.4%) Equivocal	29/37 (78.4%)
Volunteer Whole Blood Donors:			
- First-Time†	2,508	71 (2.8%) Reactive 3 (0.1%) Equivocal	60/71 (84.5%)
- Random‡	1,006	1 (0.1%) Reactive 1(0.1%) Equivocal	0/1 (0.0%)

\# Information about age and gender for individual donors is not available.

* FDA-licensed version 2.0 HCV strip immunoblot assay.

† Individuals who donated whole blood for the first time.

‡ Includes first-time donors and individuals who donated multiple times.

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SPECIFIC PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Reproducibility has not been established for EDTA, heparin, sodium citrate and potassium oxalate anticoagulated specimens. The user is responsible for establishing assay reproducibility when using these matrices.

The reproducibility of AxSYM Anti-HCV was determined in two studies. All estimates of standard deviation (SD) and percent coefficient of variation (%CV) were determined with a variance component analysis[20] for a random effects model.[21] One study used the National Committee for Clinical Laboratory Standards (NCCLS) Approved Guideline EP5-A[22] as a guide. A coded 35-member panel was composed of seven unique members repeated five times within the panel. The seven unique members were tested in duplicate twice daily over a period of 20 days using one reagent lot at three sites. Each daily run included two replicates of the assay controls. This study was designed to provide estimates of total and within-run reproducibility of AxSYM Anti-HCV during laboratory operation. While this study incorporated factors that influence reproducibility (*e.g.*, sample preparation, test material stability, carryover, and drift), their effects could not be individually estimated. The study design did not incorporate possibly significant sources of variability such as differences among operators or among lots of calibrator or reagents. The results from one site are shown in Table II.

TABLE II

AxSYM Anti-HCV Reproducibility - One Site, One Lot

Panel Members and Controls	Total No. Replicates	Grand Mean (S/CO)	Within-Run SD	Within-Run %CV	Total* SD	Total* %CV
A	80	14.21	1.019	7.2	1.042	7.3
B	80	8.75	0.593	6.8	0.647	7.4
C	80	4.77	0.358	7.5	0.360	8.0
D	80	3.12	0.322	10.3	0.326	10.4
E	80	1.04	0.093	9.0	0.112	10.8
F	80	0.65	0.053	8.1	0.061	9.3
G	80	0.23	0.022	9.5	0.024	10.4
Negative Control	80	0.23	0.034	14.3	0.037	15.8
Positive Control	80	4.34	0.303	7.0	0.323	7.4

* Total variability contains within-run, between-run, and between-day variability.

In the second study, a coded 24-member panel composed of six unique members repeated four times within the panel was used. The six unique members were tested twice daily over a period of five days using three reagent lots at three sites. Each daily run included two replicates of the assay controls (Table III).

TABLE III

AxSYM Anti-HCV Reproducibility - Three Sites, Three Lots

Panel Members and Controls	Total No. Replicates	Grand Mean (S/CO)	Between-Lot† SD	Between-Lot† %CV	Between-Site† SD	Between-Site† %CV	Total† SD	Total† %CV
AA	360	14.48	1.502	10.4	1.289	8.9	1.502	10.4
BB	360	8.40	0.899	10.7	0.756	9.0	0.901	10.7
CC	360	3.68	0.437	11.9	0.395	10.7	0.437	11.9
DD	360	1.30	0.180	13.8	0.180	13.8	0.188	14.4
EE	359‡	0.65	0.091	14.0	0.094	14.3	0.095	14.5
FF	360	0.34	0.067	20.0	0.075	22.2	0.078	23.2
Negative Control	180	0.42	0.061	14.5	0.058	13.8	0.065	15.5
Positive Control	180	4.02	0.480	11.9	0.775	19.3	0.796	19.8

* Contains within-run and between-run variability.

† Total variability contains within-run, between-run, between-lot, and between-site variability.

‡ One replicate missing due to an instrument error.

COMPARISON OF RESULTS

The data in this section are not the basis for claiming performance of AxSYM Anti-HCV in any particular population. While the data presented in Table IV for different categories of individuals, including those with characterized HCV infections, are descriptive of populations that were studied, the effect of the type of HCV infection or HCV-associated disease on the performance of AxSYM Anti-HCV is not known.

Results from the evaluation of 679 individuals appropriate for the AxSYM Anti-HCV intended use are presented in Table IV. Specimens were characterized by testing for anti-HCV with reference assays. Those specimens that contained anti-HCV were defined by reactive results with Abbott HCV EIA 2.0 (similar antigens to AxSYM Anti-HCV: core, 33c, c200, and c100-3) and with a FDA-licensed version 2.0 HCV strip immunoblot assay (c22 core, 33c, c100-3, and 5.1.1). Specimens not shown to contain anti-HCV were defined by any of the following patterns of results: HCV EIA 2.0 nonreactive, HCV EIA 2.0 reactive, and immunoblot nonreactive, or HCV EIA 2.0 reactive and immunoblot indeterminate. AxSYM Anti-HCV results were analyzed for **% Agreement Positive** (AxSYM Anti-HCV reactive results among specimens defined as containing anti-HCV) and **% Agreement Negative** (AxSYM Anti-HCV nonreactive results among specimens not shown to contain anti-HCV).

TABLE IV

% Agreement of AxSYM Anti-HCV#

Category	Number of Specimens Tested	% Agreement Positive (95% Confidence Interval)	% Agreement Negative (95% Confidence Interval)
Characterized HCV Infection*	157	157/157 (100.0%) (97.7 - 100.0)	
Individuals with Anti-HCV†	273	273/273 (100.0%) (98.7 - 100.0)	
Hospital Patients with Physicians' Orders for Hepatitis Testing	99	5/5 (100.0%) (47.8 - 100.0)	93/94 (98.9%) (94.2 - 100.0)
Individuals at Increased Risk:			
- Hemodialysis Patients	50	26/26 (100.0%) (86.8 - 100.0)	16/24‡ (66.7%) (44.7 - 84.4)
- Hemophilia Patients	50	50/50 (100.0%) (92.9 - 100.0)	
- Injection Drug Users	50	15/15 (100.0%) (78.2 - 100.0)	35/35 (100.0%) (90.0 - 100.0)

\# Information about age and gender for individual donors is not available.

* Characterized infections include 33 acute (15 seroconversions and 18 with jaundice or high aminotransferase levels and with other likely causes excluded), 24 chronic (anti-HCV detected for ≥ 6 months and one or more among histopathologic evidence of chronic hepatitis, treatment with interferon, or detectable HCV RNA), and 100 asymptomatic (identified as HCV-infected during donor screening). Tested specimens were selected from frozen collections and did not represent random samples of the studied populations.

† Insufficient information to enable determination of the state of HCV infection.

‡ AxSYM Anti-HCV results for these 24 specimens: 16 nonreactive, 6 reactive, and 2 equivocal.

AxSYM Anti-HCV % Agreement was also evaluated by testing 15 commercially available HCV seroconversion panels obtained from individuals who developed acute HCV infection. These individuals were also included in the category of Characterized HCV Infections, Table IV. Fourteen of the 15 individuals had elevated transaminase levels. Earliest AxSYM Anti-HCV reactivity coincided with that for HCV EIA 2.0 in five individuals. Reactivity was detected earlier by AxSYM Anti-HCV than by HCV EIA 2.0 in nine individuals. HCV EIA 2.0 was reactive earlier than AxSYM Anti-HCV in one individual.

HCV GENOTYPES

A study was performed to determine if AxSYM Anti-HCV could yield reactive results during infections with different variants of HCV. One hundred twenty-seven specimens representing genotypes or subtypes 1a, 1b, 2a, 2b, 3, 3a, 4, 5, and 6a were obtained from North America, Central America, Africa, and Asia. The specimens had been genotyped by investigational methods (restriction fragment length polymorphism, line probe assay, sequence analysis of 5' nontranslated or core regions, or primer-specific polymerase chain reaction). These genotyping methods are not FDA approved and their accuracy is not known. The specimens were characterized by testing for anti-HCV with reference assays (as described above for Table IV). Anti-HCV was detected in 125 of 127 specimens. Two specimens (one subtype 2b and one subtype 5) were nonreactive by AxSYM Anti-HCV and HCV EIA 2.0 and negative by a FDA-licensed version 2.0 HCV strip immunoblot assay. AxSYM Anti-HCV was reactive in 100.0% of the 125 specimens shown to contain anti-HCV. These data are suggestive of AxSYM Anti-HCV reactivity for a broad range of HCV variants.

ANALYTICAL SPECIFICITY

Two hundred potentially cross-reactive specimens were tested by AxSYM Anti-HCV (Table V). One hundred eighty-four specimens were nonreactive, one specimen was equivocal, and 15 specimens were reactive by AxSYM Anti-HCV. Thirteen of the 15 AxSYM Anti-HCV reactive specimens were positive for anti-HCV by a FDA-licensed version 2.0 HCV strip immunoblot assay.

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TABLE V
Analytical Specificity (Cross-Reactivity) of AxSYM Anti-HCV

Category	Number of Specimens Tested	AxSYM Anti-HCV Results Nonreactive	Reactive	Equivocal	Supplemental Assay Positive* (% of AxSYM Anti-HCV Reactive)
CMV Ab Positive	10	10	0	0	NT
EBV Ab Positive	10	10	0	0	NT
HIV-1 Ab Positive	10	6	4	0	4/4 (100.0%)
HSV Ab Positive	10	10	0	0	NT
HAV Ab Positive	10	10	0	0	NT
Acute HBV Infection	10	7	3	0	3/3 (100.0%)
Chronic HBV Infection	10	9	1	0	0/1 (0.0%)
Recovered HBV Infection	10	10	0	0	NT
Rubella Ab Positive	10	10	0	0	NT
Syphilis	10	10	0	0	NT
Toxoplasma Ab Positive	10	10	0	0	NT
E. coli Infection	10	10	0	0	NT
Yeast Infection	10	6	4	0	3/4 (75.0%)
Dengue Ab Positive	10	10	0	0	NT
Rheumatoid Factor Positive	10	10	0	0	NT
Elevated IgM	10	10	0	0	NT
Elevated IgG	10	10	0	0	NT
Non-Viral Liver Diseases Autoimmune Hepatitis	5	5	0	0	NT
Alcoholic Liver Disease	12	12	0	0	NT
Primary Biliary Cirrhosis	5	5	0	0	NT
Cryptogenic Cirrhosis	3	1	1	1	1/1 (100.0%)
Hepatocellular Carcinoma	5	3	2	0	2/2 (100.0%)
TOTAL	200	184	15	1	13/15 (86.7%)

* FDA-licensed version 2.0 HCV strip immunoblot assay

NT = Not Tested

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