Alinity i Anti-HCV-06

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to hepatitis C virus (anti-HCV) in human adult serum and plasma (potassium EDTA, lithium heparin, and sodium heparin) on the Alinity i analyzer.

Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with HCV (state of infection or associated disease not determined) in persons with signs and 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 Alinity i Anti-HCV for use in screening blood, plasma, or tissue donors has not been established.

Assay performance characteristics have not been established for newborns, infants, children, or populations of immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations.

SUMMARY AND EXPLANATION OF THE TEST

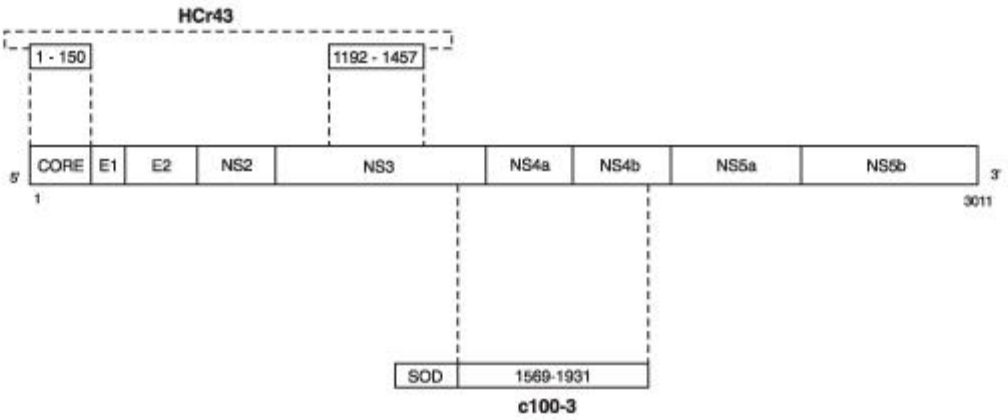
The Alinity i Anti-HCV assay is for the detection of antibodies to the hepatitis C virus (HCV). Chemiluminescent immunoassays are a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary analytes.*1* The bound analyte is detected by a series of antigen-antibody reactions. EIAs are available to identify antigens and antibodies related to viral hepatitis infection. In the Alinity i Anti-HCV final reaction, bound acridinylated conjugates are used to generate a chemiluminescent signal.

HCV is a bloodborne virus.*2*, *3* Serological studies employing EIAs for detection of antibodies to recombinant antigens of HCV have established HCV as the cause of most bloodborne*4*, *5*, *6*, *7*, *8*, *9* as well as community-acquired*10* non-A, non-B hepatitis. The presence of anti-HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and/or may be capable of transmitting HCV infection.*11*

Although the majority of infected individuals may be asymptomatic, HCV infection may develop into chronic hepatitis, cirrhosis, and/or increased risk of hepatocellular carcinoma.*12*, *13*, *14*, *15* The implementation of blood donation screening for anti-HCV by EIAs has led to a marked decline in the risk of transfusion-transmitted hepatitis.*16*, *17*

Alinity i Anti-HCV has been designed to detect antibodies to putative structural and nonstructural proteins of the HCV genome. The relationship between the recombinant HCV proteins in Alinity i Anti-HCV and the putative structural and nonstructural proteins of the HCV genome is depicted below.*18*

- HCr43: The HCr43 protein is expressed in *Escherichia coli* (*E. coli*) and is composed of two noncontiguous coding regions of the HCV genome sequence. The first region represents amino acids 1192 to 1457 (33c) of the HCV sequence. The second of the two regions represents amino acids 1 to 150 (core) of the HCV sequence. Because of the similarity of the genomic organization of the flaviviruses, it is suggested that the first sequence is from the NS3 coding region and the second sequence is from the core coding region of HCV.
- c100-3: The c100-3 antigen is a recombinant HCV protein expressed in *Saccharomyces cerevisiae* (yeast). The genomic organization of flaviviruses suggests that the cloned sequence is contained within the putative nonstructural (NS3 and NS4) regions of HCV. The c100-3 protein is a chimeric fusion protein with 154 amino acids of human superoxide dismutase (hSOD), five linker amino acids, amino acids number 1569 to 1931 of the HCV polyprotein, and the additional five amino acid linker at the carboxyl terminus.



BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the qualitative detection of anti-HCV in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, recombinant HCV antigen coated paramagnetic microparticles, and assay diluent are combined and incubated. The anti-HCV present in the sample binds to the HCV coated microparticles. The mixture is washed. Anti-human IgG/IgM acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of anti-HCV in the sample and the RLUs detected by the system optics.

The presence or absence of anti-HCV in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

If the chemiluminescent signal of the sample is greater than or equal to the cutoff signal, the sample is considered reactive for anti-HCV.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i Anti-HCV Reagent Kit 08P05

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	**08P0521**
Tests per cartridge	100

REF	08P0521
Number of cartridges per kit	2
Tests per kit	200
MICROPARTICLES	6.6 mL
CONJUGATE	6.1 mL
ASSAY DILUENT	10.4 mL

MICROPARTICLES HCV antigen (recombinant *E. coli*, recombinant yeast) coated microparticles in MES buffer. Minimum concentration: 0.14% solids. Preservatives: antimicrobial agents.

CONJUGATE Murine anti-human IgG/IgM acridinium-labeled conjugate in MES buffer with protein (bovine) additive (152 µM) and surfactant. Minimum concentration: (IgG) 8 ng/mL / (IgM) 0.8 ng/mL. Preservatives: antimicrobial agents.

ASSAY DILUENT TRIS buffer with protein (goat) additive (102.6 g/L) and surfactant. Preservatives: ProClin 300 and antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[19, 20, 21, 22]

The following warnings and precautions apply to: CONJUGATE	
	
WARNING	Contains polyethylene glycol octylphenyl ether (Triton X-405).
H319	Causes serious eye irritation.
Prevention	
P264	Wash hands thoroughly after handling.

P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.

The following warnings and precautions apply to: ASSAY DILUENT	
	
DANGER	Contains polyethylene glycol octylphenyl ether (Triton X-405).
H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- **Prior to loading on the analyzer for the first time, gently invert cartridges 30 times.**
- **Reagent cartridges cannot be inverted after the septum has been pierced by the analyzer.**
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use. Prior to loading on the analyzer for the first time, gently invert cartridges 30 times. May be used immediately after removal from 2-8°C storage.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
			upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance. May be used immediately after removal from 2-8°C storage.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i Anti-HCV assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters**, refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Specimen Types	Collection Tubes
Serum	Serum (glass and plastic)
	Serum separator (glass and plastic)
Plasma	Lithium heparin separator (plastic)
	Sodium heparin (plastic)
	Dipotassium EDTA (plastic)

Specimen Conditions

Do not use:

- heat-inactivated specimens
- pooled specimens
- grossly hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature (study performed at 20 to 23°C)	3 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

Based on guidance from CLSI GP44-A4*23*, it is recommended that if testing will be delayed longer than the maximum storage time, remove serum or plasma from the clot, red blood cells, or separator gel and store frozen (-20°C or colder).

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

Package and label specimens 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.

PROCEDURE

Materials Provided

08P05 Alinity i Anti-HCV Reagent Kit

Materials Required but not Provided

- Alinity i Anti-HCV assay file
- 08P0501 Alinity i Anti-HCV Calibrator
- 08P0510 Alinity i Anti-HCV Controls or other control material
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

Prior to loading on the analyzer for the first time, gently invert the reagent cartridge 30 times.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 70 µL
- Sample volume for each additional test from same sample cup: 20 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 20 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Anti-HCV calibrator package insert and/or Alinity i Anti-HCV control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i Anti-HCV assay.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Calibrator 1 is tested in triplicate.

A single sample of each control level must be tested every day testing performed to evaluate the assay calibration.

- Ensure that assay control values are within the ranges specified in the control package insert.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- If statistically-based quality control limits are not available, then the calibration **should not exceed a 30-day limit for recalibration frequency**.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i Anti-HCV assay is that a single sample of each control level be tested once every day testing performed.

Note: The insert ranges for the controls are not lot specific and represent the total range of values which may be generated throughout the life of the product. It is recommended that

each laboratory establish its own means and acceptable ranges which should fall within the package insert ranges. Sources of variation that can be expected include:

- Calibration
- Control lot
- Instrument
- Calibrator lot
- Reagent lot

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished **by assaying a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24, 4th ed., for general quality control recommendations.[24]

Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected test results are invalid, and these samples must be retested. For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.

RESULTS

Calculation

The Alinity i analyzer calculates results for the Alinity i Anti-HCV assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 0.074

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

Initial Alinity i Anti-HCV Results

S/CO	Instrument Interpretation	Retest Procedure
0.00 to 0.79	Nonreactive	No retest required.
0.80 to 0.99	Grayzone	Retest in duplicate.
≥ 1.00	Reactive	No retest required.

Alinity i Anti-HCV Results

Initial Result	Retest Result	Result	Interpretation
Nonreactive	No retest required.	Nonreactive	Antibodies to HCV not detected; does not exclude the possibility of exposure to HCV.
Grayzone	Both of the duplicate retests are reactive.	Reactive	Presumptive evidence of antibodies to HCV; follow CDC recommendations25 for supplemental testing.
	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 or follow CDC recommendations25 for supplemental testing.
	Both of the duplicate retests are nonreactive.	Nonreactive	Antibodies to HCV not detected; does not exclude the possibility of exposure to HCV.
Reactive	No retest required.	Reactive	Presumptive evidence of antibodies to HCV; follow CDC recommendations25 for supplemental testing.

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 Alinity ci-series Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

· For diagnostic purposes, results should be used in conjunction with patient history and

other hepatitis markers for diagnosis of acute and chronic infection.

- 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.
- 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 recombinant antigens used in this assay.
- Immunocompromised patients who have HCV may produce levels of antibody below the sensitivity of this assay and may not be detected as positive.
- The affinity or avidity differences of anti-human IgG/IgM for anti-HCV have not been determined with this assay. Therefore, there may not be a demonstration of a significant increase in antibody level between acute and convalescent specimens for a patient in the late acute stage of infection when IgM antibodies are decreasing.
- Results obtained with the Alinity i Anti-HCV assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Assay performance characteristics have not been established for newborns, infants, children, or populations of immunocompromised or immunosuppressed patients.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[26] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- A reactive anti-HCV result does not exclude co-infection by another hepatitis virus.
- The magnitude of an Alinity i Anti-HCV assay result cannot be correlated to an end point titer.
- Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert for specimen limitations.

EXPECTED RESULTS

This study was performed on the ARCHITECT i System.

Due to geographic locations or demographics, assay results obtained in individual laboratories may vary from data presented.

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

Of the 2027 specimens tested in the ARCHITECT Anti-HCV clinical study, 1310 (64.63%) were from individuals with increased risk of HCV infection. All 1310 were at risk for HCV due to lifestyle, behavior, occupation, or a known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. Testing of these specimens was performed at three clinical sites located in Galveston, TX; Hershey, PA; and Milwaukee, WI.

The asymptomatic population (n=1310) consisted of the following race/ ethnic groups:

- 589 (44.96%) Caucasian
- 522 (39.85%) African-American
- 165 (12.60%) Hispanic
- 10 (0.76%) Asian
- 9 (0.69%) American Indian/Alaska Native
- 15 (1.15%) Other

The 1310 specimens from the asymptomatic population were obtained from the following collection locations:

- 707 (53.97%) from Galveston, TX
- 185 (14.12%) from High Point, NC
- 103 (7.86%) from Plymouth, MA
- 76 (5.80%) from Colton, CA
- 64 (4.89%) from Dallas, TX
- 56 (4.27%) from St. Petersburg, FL
- 56 (4.27%) from Miami, FL
- 44 (3.36%) from Denver, CO
- 19 (1.45%) from Chicago, IL

A total of 237 (18.09%) of the specimens in the asymptomatic population were reactive in the ARCHITECT Anti-HCV assay. The number of ARCHITECT Anti-HCV reactive results observed for the asymptomatic population at each collection location was:

- 132 of 707 (18.67%) from Galveston, TX
- 10 of 185 (5.41%) from High Point, NC
- 32 of 103 (31.07%) from Plymouth, MA
- 1 of 76 (1.32%) from Colton, CA
- 15 of 64 (23.44%) from Dallas, TX
- 10 of 56 (17.86%) from Miami, FL
- 10 of 56 (17.86%) from St. Petersburg, FL
- 13 of 44 (29.55%) from Denver, CO
- 14 of 19 (73.68%) from Chicago, IL

Of the 1310 specimens, 864 (65.95%) were female and 446 (34.05%) were male. The age was not reported for three specimens. Of the remaining 1307 specimens, the mean age was 40 years (age range: 18 to 73 years). The distribution of ARCHITECT Anti-HCV reactive, equivocal, and nonreactive results among the asymptomatic population by age and gender (n=1307) is summarized in the following table.

Age Group (Years)	Gender	ARCHITECT Anti-HCV Results			Total
		Reactive n (%)	Equivocal n (%)	Nonreactive n (%)	
0-17	F	0 (0.00)	0 (0.00)	0 (0.00)	0
	M	0 (0.00)	0 (0.00)	0 (0.00)	0
18-29	F	12 (5.58)	1 (0.47)	202 (93.95)	215
	M	8 (8.99)	0 (0.00)	81 (91.01)	89
30-39	F	15 (7.21)	1 (0.48)	192 (92.31)	208
	M	18 (18.56)	1 (1.03)	78 (80.41)	97
40-49	F	48 (18.25)	0 (0.00)	215 (81.75)	263
	M	55 (37.16)	0 (0.00)	93 (62.84)	148
50-59	F	26 (18.98)	0 (0.00)	111 (81.02)	137
	M	47 (51.09)	0 (0.00)	45 (48.91)	92
60-69	F	1 (3.03)	0 (0.00)	32 (96.97)	33
	M	4 (25.00)	0 (0.00)	12 (75.00)	16
70-79	F	1 (16.67)	0 (0.00)	5 (83.33)	6
	M	1 (33.33)	0 (0.00)	2 (66.67)	3
Total		**236 (18.06)**	**3 (0.23)**	**1068 (81.71)**	**1307**[†]

[†]Age was not reported for three subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios. Some performance characteristics for this assay were established using the ARCHITECT i System.

Alinity i Analyzer Specific Studies

The following results were generated using the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) EP05-A3.[27] Testing was conducted using 1 lot of the Alinity i Anti-HCV Reagent Kit,
1 lot of the Anti-HCV calibrator, and 1 lot of the Anti-HCV controls and 1 instrument. Two controls and 3 panels were assayed in 3 replicates at 2 separate times per day on 12 different days.

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory Precision (Total)[a]	
			SD	%CV	SD	%CV
Negative Control	72	0.13	0.005	NA[b]	0.005	NA[b]
Positive Control	72	4.31	0.133	3.1	0.138	3.2
High Negative Panel	72	0.88	0.025	2.8	0.025	2.8
Low Positive Panel	72	1.25	0.040	3.2	0.040	3.2
Moderate Positive Panel	72	2.70	0.081	3.0	0.086	3.2

[a] Includes within-run, between-run, and between-day variability.

[b] NA = not applicable

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and CLSI EP15-A2.[28, 29] Testing was conducted at 3 clinical sites using 1 lot of the Alinity i Anti-HCV Reagent Kit, 1 lot of the Alinity i Anti-HCV Calibrator, and 1 lot of the Alinity i Anti-HCV Controls and 1 instrument per site. Two controls and 3 panels were assayed in replicates of 4 at 2 separate times per day for 5 days.

Sample	n	Mean S/CO	Within-Run	Within-Day[a]	Within-Laboratory Precision (Total)[b]	Precision with Additional Component of Between-Site[c]

			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	120	0.13	0.005	NA[d]	0.005	NA[d]	0.008	NA[d]	0.009	NA[d]
Positive Control	120	4.03	0.121	3.0	0.124	3.1	0.128	3.2	0.220	5.5
High Negative Panel	120	0.74	0.022	3.0	0.023	3.1	0.026	3.5	0.046	6.3
Low Positive Panel	120	1.07	0.038	3.6	0.039	3.7	0.042	3.9	0.070	6.5
Moderate Positive Panel	120	2.36	0.077	3.3	0.083	3.5	0.086	3.7	0.154	6.5

[a] Includes within-run and between-run variability.

[b] Includes within-run, between-run, and between-day variability.

[c] Includes within-run, between-run, between-day, and between-site variability.

[d] Not applicable

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 22 seroconversion panels obtained from commercial vendors were tested on the Alinity i analyzer using the Alinity i Anti-HCV assay. The panel results were evaluated against the comparator assay (ARCHITECT Anti-HCV) and data are summarized in the following table.

	Days to Anti-HCV First Reactive Result from Initial Draw Date		Difference in Days to Anti-HCV First Reactive Result (Alinity - ARCHITECT)
Panel ID	Alinity i Anti-HCV	ARCHITECT Anti-HCV	
6212	12	14	-2
6213	37	37	0
6216	23	23	0
6222	40	40	0
6225	78	78	0

Panel ID	Days to Anti-HCV First Reactive Result from Initial Draw Date		Difference in Days to Anti-HCV First Reactive Result (Alinity - ARCHITECT)
	Alinity i Anti-HCV	ARCHITECT Anti-HCV	
6226	37	37	0
6228	28	31	-3
6229	17	17	0
9044	25	25	0
9045	37	37	0
9047	28	28	0
9054	77	77	0
9058	10	10	0
10041	6	6	0
10071	77	77	0
10165	24	24	0
PHV904	9	9	0
PHV906	0	0	0
PHV912	7	7	0
PHV920	13	13	0
PHV922	3	3	0
PHV913	7	7	0

Percent Agreement

A study was performed to compare the anti-HCV assay on the Alinity i analyzer and the ARCHITECT i2000SR system using 1 lot each of the Anti-HCV Reagent Kit, Anti-HCV Calibrator, and Anti-HCV Controls. Of the 210 specimens/samples tested, 101 were nonreactive, 9 were grayzone, and 100 were reactive based on the ARCHITECT Anti-HCV results on the ARCHITECT i2000SR instrument. An aliquot of each specimen/sample was tested on
1 Alinity i analyzer at each of the 3 clinical testing sites and on 1 ARCHITECT i2000SR instrument at 1 clinical testing site.

Site	Alinity i	ARCHITECT Anti-HCV	Negative %	Positive %

	Anti-HCV	Reactive	Grayzone	Nonreactive	Agreement (95% Confidence Interval)[a]	Agreement (95% Confidence Interval)[a]
1	Reactive	95	0	0	96.04 (97/101)	95.00 (95/100)
	Grayzone	4	4	4	(90.26, 98.45)	(88.82, 97.85)
	Nonreactive	1	5	97		
2	Reactive	100	4	0	96.04 (97/101)	100.00 (100/100)
	Grayzone	0	4	4	(90.26, 98.45)	(96.30, 100.00)
	Nonreactive	0	1	97		
3	Reactive	100	5	0	94.06 (95/101)	100.00 (100/100)
	Grayzone	0	3	6	(87.64, 97.25)	(96.30, 100.00)
	Nonreactive	0	1	95		
All	Reactive	295	9	0	95.38 (289/303)	98.33 (295/300)
	Grayzone	4	11	14	(92.39, 97.23)	(96.16, 99.29)
	Nonreactive	1	7	289		

[a] 95% confidence intervals for negative percent agreement and positive percent agreement were estimated using Wilson score method.

$$\text{Positive Percent Agreement} = \frac{\text{[Number of initially reactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of initially reactive ARCHITECT i2000SR results]}} \times 100\%$$

$$\text{Negative Percent Agreement} = \frac{\text{[Number of initially nonreactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of initially nonreactive ARCHITECT i2000SR results]}} \times 100\%$$

ARCHITECT i2000/ i2000SR System Specific Studies

The following results were generated using the ARCHITECT i2000 / i2000SR System.

Clinical Performance

A prospective multi-center study was conducted to evaluate the ability of the ARCHITECT Anti-HCV assay to detect anti-HCV antibodies in a group of individuals that would normally be tested in a clinical situation. Of the 2027 specimens tested in the ARCHITECT Anti-HCV clinical study, 1310 specimens were obtained from individuals with increased risk of HCV infection due to lifestyle, behavior, occupation, disease state, or a known exposure event and 717 specimens were obtained from individuals exhibiting signs and symptoms of hepatitis infection.

The specimen population (n=2027) consisted of the following race/ethnic groups:

- 1035 (51.06%) Caucasian
- 631 (31.13%) African-American
- 294 (14.50%) Hispanic
- 30 (1.48%) Asian
- 12 (0.59%) American Indian/Alaska Native
- 25 (1.23%) Other

The 2027 specimens from the specimen population were obtained from the following collection locations:

- 757 (37.35%) from Galveston, TX
- 345 (17.02%) from Plymouth, MA
- 185 (9.13%) from High Point, NC
- 181 (8.93%) from Chicago, IL
- 140 (6.91%) from Denver, CO
- 126 (6.22%) from Dallas, TX
- 118 (5.82%) from Colton, CA
- 94 (4.64%) from Miami, FL
- 81 (4.00%) from St. Petersburg, FL

Of the 2027 specimens, 1126 (55.55%) were female and 901 (44.45%) were male. The age was not reported for 3 specimens. Of the remaining 2024 specimens, the mean age was 41 years (age range: 18 to 83 years). The HCV status was determined for each specimen using the comparator anti-HCV assay and, as indicated, supplemental assays (Chiron RIBA HCV 3.0 Strip Immunoblot Assay [SIA] and Roche COBAS AMPLICOR Hepatitis C Virus [HCV] Test v2.0). During the clinical study, all comparator and supplemental testing was performed following manufacturers' instructions. Each specimen was also tested using the ARCHITECT Anti-HCV assay at the 3 clinical sites located in Galveston, TX; Hershey, PA; and Milwaukee, WI.

Results by Specimen Classification

Following testing with the comparator anti-HCV assay and supplemental testing, where indicated, 2027 specimens were assigned an HCV status of *HCV Infected*, *HCV Not Determined*, or *HCV Not Infected* based on the final results obtained with the comparator or supplemental assays according to the following algorithm.

Comparator Anti-HCV Assay Final Result	Supplemental Assay Final Results		HCV Status[25]
Nonreactive	--		Not Infected[a]
	Chiron RIBA HCV 3.0 SIA	**Roche COBAS AMPLICOR HCV Test v2.0**	
Reactive	Positive	--	Infected[b]
Reactive	Indeterminate or Negative	Positive	Infected[c]
Reactive	Indeterminate or Negative	Equivocal	Not Determined[d]
Reactive	Indeterminate or Negative	Negative	Not Infected[a]
Equivocal	Positive	--	Infected[b]
Equivocal	Indeterminate or Negative	Positive	Infected[c]
Equivocal	Indeterminate or Negative	Equivocal	Not Determined[d]
Equivocal	Indeterminate or Negative	Negative	Not Infected[a]

[a] A negative test result does not exclude the possibility of exposure to HCV.

[b] State of associated disease Not Determined.

[c] Indicates active HCV infection.

[d] HCV status cannot be determined.

-- = not performed

Comparison of Results

The following table compares the ARCHITECT Anti-HCV assay results with HCV status for the increased risk and signs and symptoms populations. The increased risk population was ranked according to the risk of HCV infection in study subjects. The risk of HCV infection was ranked based on a clinical evaluation of the likelihood of acquiring HCV through each mode of transmission: the mode of transmission was ranked higher if the likelihood of acquiring HCV was greater.[30] Each specimen was assigned only one risk (the highest ranked). The status of HCV infection was assigned according to the algorithm presented in the table in the previous section. Of the 2027 specimens tested, the status of 616 specimens was *HCV Infected*. The status of 1411 specimens was *HCV Not Infected*. No specimens had the status *HCV Not Determined*. The data are summarized in the following table.

Specimen Population	HCV Status						Total n (%)
	HCV Infected			Not HCV Infected			
	Reactive n (%)	Equivocal n (%)	Nonreactive n (%)	Reactive n (%)	Equivocal n (%)	Nonreactive n (%)	
Individuals with increased risk of HCV infection							
Recipients of clotting factor concentrates prior to 1987	1 (0.05)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.10)	3 (0.15)
Users of injecting drugs	97 (4.79)	0 (0.00)	0 (0.00)	8 (0.39)	0 (0.00)	85 (4.19)	190 (9.37)
Multiple sex partners	53 (2.61)	0 (0.00)	1 (0.05)	6 (0.30)	1 (0.05)	587 (28.96)	648 (31.97)
Transfusion recipient prior to July 1992 or received blood from donor later to be found HCV positive	18 (0.89)	0 (0.00)	0 (0.00)	2 (0.10)	0 (0.00)	22 (1.09)	42 (2.07)
Perinatal exposure; mother was infected with hepatitis C	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.15)	3 (0.15)
Men who have sex with men	1 (0.05)	0 (0.00)	0 (0.00)	2 (0.10)	0 (0.00)	11 (0.54)	14 (0.69)
Needle stick or mucosal exposure on the job	13 (0.64)	0 (0.00)	0 (0.00)	3 (0.15)	2 (0.10)	252 (12.43)	270 (13.32)
Other[a]: Household contact with hepatitis C infected individual and/or intranasal cocaine user	31 (1.53)	0 (0.00)	0 (0.00)	2 (0.10)	0 (0.00)	107 (5.28)	140 (6.91)
Individuals with signs and symptoms of a hepatitis infection	400 (19.73)	0 (0.00)	1 (0.05)	7 (0.35)	0 (0.00)	309 (15.24)	717 (35.37)
Total	**614 (30.29)**	**0 (0.00)**	**2 (0.10)**	**30 (1.48)**	**3 (0.15)**	**1378 (67.98)**	**2027 (100.00)**

[a] Not based on CDC recommendations.

Percent Agreement

The positive percent agreement between the ARCHITECT Anti-HCV assay results and the *HCV Infected* status for the overall population (n = 2027) was 99.68% (614/616, with a 95% confidence interval of 98.83% to 99.96%). Among the specimens with *HCV Infected* status, there were 0.00% (0/616) equivocal results by ARCHITECT Anti-HCV assay (95% confidence interval is 0.00% to 0.60%). The negative percent agreement between the ARCHITECT Anti-HCV assay results and the *HCV Not Infected* status for the overall population was 97.66% (1378/1411, with a 95% confidence interval of 96.73% to 98.38%). Among the specimens with *HCV Not Infected* status, there were 0.21% (3/1411) equivocal results by ARCHITECT Anti-HCV assay (95% confidence interval is 0.04% to 0.62%).

The table below summarizes the positive percent agreement and negative percent agreement data for individuals with increased risk of HCV infection by hepatitis risk group.

Hepatitis C Ranked Risk Group	Positive Percent Agreement % (x/n)[b]	95% Confidence Interval	Negative Percent Agreement % (x/n)[b]	95% Confidence Interval
Recipients of clotting factor concentrates prior to 1987	100.00 (1/1)	2.50 - 100.00	100.00 (2/2)	15.81 - 100.00
Users of injecting drugs	100.00 (97/97)	96.27 - 100.00	91.40 (85/93)	83.75 - 96.21
Multiple sex partners	98.15 (53/54)	90.11 - 99.95	98.82 (587/594)	97.59 - 99.52
Transfusion recipient prior to July 1992 or received blood from donor later to be found HCV positive	100.00 (18/18)	81.47 - 100.00	91.67 (22/24)	73.00 - 98.97
Perinatal exposure; mother was infected with hepatitis C	NA (0/0)	NA	100.00 (3/3)	29.24 - 100.00
Men who have sex with men	100.00 (1/1)	2.50 - 100.00	84.62 (11/13)	54.55 - 98.08
Needle stick or mucosal exposure on the job	100.00 (13/13)	75.29 - 100.00	98.05 (252/257)	95.52 - 99.37
Other[a]: Household contact with hepatitis C infected individual and/or intranasal cocaine user	100.00 (31/31)	88.78 - 100.00	98.17 (107/109)	93.53 - 99.78
Total	**99.53 (214/215)**	**97.44 - 99.99**	**97.63 (1069/1095)**	**96.54 - 98.44**

[a] Not based on CDC recommendations.

[b] x = the number of reactive (or nonreactive) ARCHITECT Anti-HCV results that were in agreement with the HCV status as determined by comparator or supplemental testing;

n = the total number of HCV Infected status (or HCV Not Infected status) results as determined by comparator or supplemental testing.

$$\text{Positive Percent Agreement} = \frac{\text{[Number of ARCHITECT Anti-HCV reactive results with the HCV Infected status]}}{\text{[Total number of HCV Infected status]}} \times 100\%$$

$$\text{Negative Percent Agreement} = \frac{\text{[Number of ARCHITECT Anti-HCV nonreactive results with the HCV Not Infected status]}}{\text{[Total number of HCV Not Infected status]}} \times 100\%$$

Analytical Specificity

The ARCHITECT Anti-HCV assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HCV infection. The specimens were tested using the ARCHITECT Anti-HCV assay and the comparator anti-HCV assay. The final results for each of the specimens were compared between the 2 assays. The reactivity of the ARCHITECT Anti-HCV assay in individuals with medical conditions unrelated to HCV infection is summarized in the following table.

Reactivity of the ARCHITECT Anti-HCV Assay in Individuals with Medical Conditions Unrelated to HCV Infection

		Comparator Anti-HCV Assay								
		Nonreactive			**Equivocal**			**Reactive[a]**		
		ARCHITECT Anti-HCV			**ARCHITECT Anti-HCV**			**ARCHITECT Anti-HCV**		
Category	**n**	**NR[b]**	**EQ[b]**	**R[b]**	**NR[b]**	**EQ[b]**	**R[b]**	**NR[b]**	**EQ[b]**	**R[b]**
Cytomegalovirus (anti-CMV positive)	10	10	0	0	0	0	0	0	0	0
Epstein-Barr Virus (anti-EBV positive)	10	10	0	0	0	0	0	0	0	0
Hepatitis A Virus (anti-HAV positive)	10	8	0	0	1[c]	0	0	0	0	1
Hepatitis B Virus (anti-HBV positive)	10	10	0	0	0	0	0	0	0	0
Human Immunodeficiency Virus (anti-HIV-1 positive)	10	6	0	0	0	0	0	1[d]	0	3
Anti-Nuclear Antibody (ANA)	10	10	0	0	0	0	0	0	0	0
Escherichia coli (*E. Coli*)	3	3	0	0	0	0	0	0	0	0
Elevated IgG	10	9	0	0	0	0	0	0	0	1
Elevated IgM	10	8	0	0	0	0	0	0	0	2
Elevated total	10	4	0	0	0	0	0	0	0	6

		Comparator Anti-HCV Assay								
		Nonreactive			Equivocal			Reactive[a]		
		ARCHITECT Anti-HCV			ARCHITECT Anti-HCV			ARCHITECT Anti-HCV		
Category	**n**	**NR[b]**	**EQ[b]**	**R[b]**	**NR[b]**	**EQ[b]**	**R[b]**	**NR[b]**	**EQ[b]**	**R[b]**
bilirubin										
Elevated total protein	8	5	0	0	0	0	0	0	0	3
Herpes Simplex Virus (HSV) IgG	5	5	0	0	0	0	0	0	0	0
Human T-cell Lymphotropic Virus (HTLV)	10	10	0	0	0	0	0	0	0	0
Human Anti-Mouse Antibodies (HAMA) positive	10	10	0	0	0	0	0	0	0	0
Influenza vaccine recipients	10	9	0	0	0	0	0	0	0	1
Multiparous female	10	10	0	0	0	0	0	0	0	0
Non-viral liver disease	10	10	0	0	0	0	0	0	0	0
Rheumatoid factor positive	10	10	0	0	0	0	0	0	0	0
Rubella	10	10	0	0	0	0	0	0	0	0
Syphilis	10	6	0	0	0	0	0	1[e]	0	3
Systemic Lupus Erythematosus (SLE)	4	4	0	0	0	0	0	0	0	0
Toxoplasmosis IgG positive	9	9	0	0	0	0	0	0	0	0
Varicella Zoster Virus (VZV) positive	10	9	0	0	0	0	0	0	0	1
Yeast infection	9	9	0	0	0	0	0	0	0	0
Total	**218**	**194**	**0**	**0**	**1**	**0**	**0**	**2**	**0**	**21**

[a] Each reactive anti-HCV result was verified using the comparator anti-HCV assay.

[b] NR = Nonreactive, EQ = Equivocal, R = Reactive

[c] The final result of the anti-HAV positive specimen was anti-HCV negative when tested using the Chiron RIBA HCV 3.0 SIA and HCV RNA negative when tested using the Roche COBAS AMPLICOR HCV Test v2.0.

[d] The final result of the anti-HIV-1 positive specimen was anti-HCV indeterminate when tested using the Chiron RIBA HCV 3.0 SIA and HCV RNA negative when tested using the Roche COBAS AMPLICOR HCV Test v2.0.

[e] The final result of the syphilis specimen was anti-HCV negative when tested using the Chiron RIBA HCV 3.0 SIA and HCV RNA negative when tested using the Roche COBAS

AMPLICOR HCV Test v2.0.

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), hemoglobin, total protein, and triglycerides showed less than 10% interference in the ARCHITECT Anti-HCV assay for high negative samples (S/CO range: 0.60 to 0.99) and low positive samples (S/CO range: 1.00 to 1.40):

Potentially Interfering Substance	Interferent Level
Bilirubin	≤ 20 mg/dL
Hemoglobin	≤ 500 mg/dL
Total Protein	≤ 12 g/dL
Triglycerides	≤ 3000 mg/dL

Tube Type Matrix Comparison

The following tube types are acceptable for use:

- Glass: serum and serum separator
- Plastic: serum, serum separator, lithium heparin plasma separator, sodium heparin, and dipotassium EDTA

On average, the tube types listed in the table below showed less than a 10% difference when compared to the control tube type (glass serum) for high negative samples (S/CO range: 0.60 to 0.99) and low positive samples (S/CO range: 1.00 to 1.40).

With the tube types listed in the table below, the ARCHITECT Anti-HCV assay showed the following distribution of percent differences when compared to the glass serum tube type.

	Distribution of the Differences		
Tube Type	**< 10%**	**≥ 10% to ≤ 20%**	**> 20%**
Glass Serum Separator	85.0% (34/40)	15.0% (6/40)	-
Plastic Serum	95.0% (38/40)	5.0% (2/40)	-
Plastic Serum Separator	90.0% (36/40)	7.5% (3/40)	2.5% (1/40)
Plastic Lithium Heparin Plasma Separator	72.5% (29/40)	22.5% (9/40)	5.0% (2/40)

Tube Type	Distribution of the Differences		
	< 10%	≥ 10% to ≤ 20%	> 20%
Plastic Sodium Heparin	75.0% (30/40)	22.5% (9/40)	2.5% (1/40)
Plastic Dipotassium EDTA	72.5% (29/40)	20.0% (8/40)	7.5% (3/40)

Genotype Detection

The ARCHITECT Anti-HCV assay detects the same commonly recognized genotypes of HCV as the comparator anti-HCV assay. Two lots of genotype panels were obtained from Teragenix Corporation which consisted of the following genotypes, as determined by the vendor: 1a, 1b, 1c, 2, 2a, 2b, 2c, 3a, 3b, 4, 4a, 4c, 4d, 5, 5a, and 6a. The lots were tested using the ARCHITECT Anti-HCV assay and the comparator anti-HCV assay, and the final results were compared. The ARCHITECT and the comparator anti-HCV assay final results were in 100% agreement for the genotypes of HCV.

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