Alinity i

Anti-HCV Reagent Kit

en
Anti-HCV
08P06
G71272R02
B8P060

Revised March 2017.

REF 08P0622
REF 08P0632

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NAME

Alinity i Anti-HCV Reagent Kit

INTENDED USE

The Alinity i Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibody to Hepatitis C virus (anti-HCV) in human serum and plasma, including specimens collected post mortem (non-heart beating) on the Alinity i analyzer.

The Alinity i Anti-HCV assay is intended to be used as an aid in the diagnosis of Hepatitis C infection and as a screening test to prevent transmission of Hepatitis C virus (HCV) to recipients of blood, blood components, cells, tissue and organs.

SUMMARY AND EXPLANATION OF THE TEST

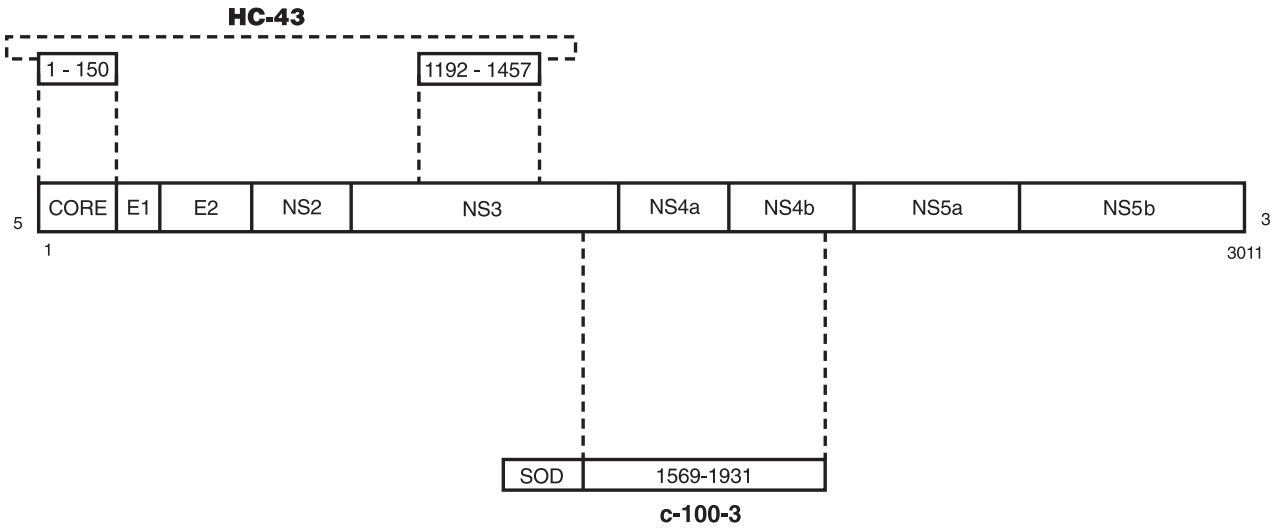
The Alinity i Anti-HCV assay is for the detection of antibodies to hepatitis C virus (HCV). Chemiluminescent immunoassays are a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary 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[4-9] 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.[12-15] 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.

Hepatitis C antigens HCr43 and c100-3 are prepared under US license by Chiron Corporation under a shared manufacturing agreement. The Alinity i Anti-HCV assay is manufactured under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.



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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 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 direct 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.

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 08P06

NOTE: Some kit sizes are not available in all countries. Please contact your local distributor.

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

REF	08P0622	08P0632
Tests per cartridge	100	500
Number of cartridges per kit	2	2
Tests per kit	200	1000
MICROPARTICLES	6.6 mL	27.0 mL
CONJUGATE	6.1 mL	26.5 mL
ASSAY DILUENT	10.4 mL	47.1 mL

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

CONJUGATE murine anti-IgG/anti-IgM acridinium-labeled conjugate in MES buffer. Minimum concentration: (IgG) 8 ng/mL/(IgM) 0.8 ng/mL. Preservatives: antimicrobial agents.

ASSAY DILUENT TRIS buffer with protein stabilizers. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

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.[19-22]

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.

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

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 cartridge 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 cartridge 30 times.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Reagent cartridges cannot be inverted after the septum has been pierced by the analyzer. Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance.

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 or 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 on the ARCHITECT i System.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Potassium EDTA
	Lithium heparin
	Sodium heparin
	Sodium citrate
	ACD
	CPDA-1
	CPD
	CP2D
	Potassium oxalate

- Performance has been established for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) that have been collected up to 15 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens.[23]
- Testing of cadaveric blood specimens from patients with plasma dilution due to transfusions of > 2000 mL of blood or colloids within 48 hours, or > 2000 mL of crystalloids within 1 hour (or any combination thereof) prior to collection of the specimens have not been validated.
- For cadaveric donors, serum and plasma may be used; follow general standards and/or regulations for collection, storage and handling.
- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
- 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. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.
- 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.
- 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.

Prepare cadaveric blood specimens as follows:

- After initial centrifugation, recentrifuge specimens as described below.
- If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{\text{100 000 g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

$$RCF = 1.12 \times r_{max} \, (rpm/1000)^2$$

RCF - The relative centrifugal force generated during centrifugation.

rpm - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).

Centrifugation Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.

r_{max} - Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (rmax) should be manually measured in millimeters and the RCF calculated.

g-minutes - The unit of measure for the product of RCF (× g) and centrifugation time (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 storage conditions were verified on the ARCHITECT i System.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/ Plasma	2 to 8°C	7 days	Specimens may be stored on or off the clot or red blood cells.
Cadaveric	Room temperature (15 to 30°C)	3 days	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.
	2 to 8°C	7 days	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.

Specimens may be stored for up to 7 days refrigerated at 2-8°C prior to being tested. If testing will be delayed more than 7 days, the specimens should be removed from the clot, red blood cells, or separator gel and stored frozen (-20°C or colder).

Avoid more than 6 freeze/thaw cycles for serum/plasma.

No qualitative differences were observed for cadaveric blood specimens (nonreactive or spiked reactive) when subjected to up to 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.

PROCEDURE

Materials Provided

08P06 Alinity i Anti-HCV Reagent Kit

Materials Required but not Provided

- Alinity i Anti-HCV assay file
- 08P0601 Alinity i Anti-HCV Calibrator
- 08P0610 Alinity i Anti-HCV Controls or other control material
- 06P1160 Alinity Trigger Solution
- 06P1265 Alinity Pre-Trigger Solution
- 06P1368 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 controls 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.

Each assay control must be tested to evaluate the assay calibration.

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 24 hours each day of use.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

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-A3 or other published guidelines, for general quality control recommendations.[24]

- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary. For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[25]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

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 Results

S/CO	Instrument Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required.
≥ 1.00	Reactive	Retest in duplicate.

Duplicate Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered nonreactive for anti-HCV.
One or both results reactive	Specimen considered repeatedly reactive for anti-HCV.

Repeatedly reactive anti-HCV specimens should be investigated further in supplemental tests such as other HCV-specific immunoassays and immunoblot assays or a combination thereof and/or NAT tests.

NOTE: For details on configuring the Alinity i analyzer to use grayzone and high reactive interpretations, refer to the Alinity ci-series Operations Manual, Section 2. The grayzone and high reactive interpretations are editable parameters, and should be utilized per end user requirements.

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

- False positive results can be expected with any test kit. The proportion of these falsely reactive specimens is dependent upon the specificity of the test kit, specimen integrity, and on the prevalence of HCV antibodies in the population being screened.
- If the Alinity i Anti-HCV results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute or chronic infection.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.

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.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[26] Testing was conducted using 3 lots of the Alinity i Anti-HCV Reagent Kit, 3 lots of the Alinity i Anti-HCV Calibrator, and 3 lots of the Alinity i Anti-HCV Controls and 1 instrument. Two controls and 3 human plasma panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	n	Mean (S/CO)	SD	%CV	SD (Range[b])	%CV (Range[b])
Negative Control	358	0.06	0.009	16.2	0.011 (0.011-0.011)	18.8 (16.4-22.2)
Positive Control	356	3.83	0.143	3.7	0.205 (0.152-0.279)	5.4 (3.9-7.6)
Panel 1	360	0.75	0.030	4.0	0.041 (0.036-0.049)	5.5 (4.6-7.0)
Panel 2	357	1.29	0.049	3.8	0.064 (0.054-0.080)	5.0 (4.0-6.6)
Panel 3	360	3.10	0.128	4.1	0.168 (0.133-0.222)	5.4 (4.2-7.5)

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

[b] Maximum and minimum SD or %CV for each reagent lot and instrument combination.

Specificity

A total of 5123 specimens from blood donors and hospitalized patients (HP) were tested using the Alinity i Anti-HCV assay and a commercially-available Anti-HCV assay. Repeatedly reactive specimens were further tested by supplemental testing.

Of the 10 initially reactive donor specimens, 7 were repeatedly reactive and confirmed negative by supplemental testing.

Of the 25 initially reactive hospitalized patient specimens, 16 specimens were confirmed positive, 6 specimens were negative, and 3 specimens were unable to be categorized.

		Alinity i Anti-HCV				Commercially-Available Anti-HCV Assay
Category	n	IR (% of Total)	RR (% of Total)	Number Positive by Supplemental Testing (% of RR)	Specificity [a] (95% CI)	Specificity [a] (95% CI)
Blood Donors - Serum[b]	2646	6 (0.23)	5 (0.19)	0 (0.00)	99.81% (2641/2646) (99.56 - 99.94)	99.85% (2642/2646) (99.61 - 99.96)
Blood Donors - Plasma	2477	4 (0.16)	2 (0.08)	0 (0.00)	99.92% (2475/2477) (99.71 - 99.99)	99.92% (2475/2477) (99.71 - 99.99)
Total Donors[b]	5123	10 (0.20)	7 (0.14)	0 (0.00)	99.86% (5116/5123) (99.72 - 99.95)	99.88% (5117/5123) (99.75 - 99.96)
Hospitalized Patients	695	25 (3.60)	25 (3.60)	16 (64.00)	99.11% (670/676) (98.08 - 99.67)	99.11% (670/676) (98.08 - 99.67)

IR = Initially Reactive, RR = Repeatedly Reactive, CI = Confidence Interval

[a] Repeatedly reactive specimens determined to be positive by supplemental testing or unable to be categorized were excluded from these calculations.

[b] A repeatedly reactive specimen observed on the Alinity i Anti-HCV assay showed discrepant results between the Alinity i Anti-HCV assay (1.47 S/CO, 1.17 S/CO, and 1.08 S/CO) and the commercially-available anti-HCV assay (0.99 S/CO).

Sensitivity

Anti-HCV Positive, Acute and Chronic HCV Infection, and Genotypes 1 to 6

A total of 459 confirmed positive specimens were evaluated using the Alinity i Anti-HCV assay and a commercially-available Anti-HCV assay.

Specimen Category	n	Number RR	Alinity i Anti-HCV Sensitivity	Commercially-Available Anti-HCV Assay Sensitivity
Anti-HCV Positive	302	302	100.00% (302/302)	100.00% (302/302)
Acute HCV Infection	28	28	100.00% (28/28)	100.00% (28/28)
Chronic HCV Infection	22	22	100.00% (22/22)	100.00% (22/22)
Genotype 1	22	22	100.00% (22/22)	100.00% (22/22)
Genotype 2	24	24	100.00% (24/24)	100.00% (24/24)
Genotype 3	23	23	100.00% (23/23)	100.00% (23/23)
Genotype 4 (Including non-a subtype)	23	23	100.00% (23/23)	100.00% (23/23)
Genotype 5	12	12	100.00% (12/12)	100.00% (12/12)
Genotype 6	3	3	100.00% (3/3)	100.00% (3/3)
Total	459	459	100.00% (459/459)	100.00% (459/459)

RR = Repeatedly Reactive

The overall sensitivity was estimated in these studies to be 100.00% (459 /459) with a 95% confidence interval of 99.20 to 100.00%.

Chronic HCV Infection, Anti-HCV/HCV RNA Positive, and Increased Risk for HCV Infection

This study was performed on the ARCHITECT i System.

A total of 117 specimens were tested using the ARCHITECT Anti-HCV assay. Repeatedly reactive samples were further tested by supplemental testing. Of the 117 specimens, 100 were repeatedly reactive, and were anti-HCV positive by supplemental testing.

Specimen Category	n	ARCHITECT Anti-HCV Number Repeatedly Reactive (% of Total)	Number of Positive by Supplemental Testing[a] (% of Repeatedly Reactive)
Chronic HCV Infection	50	50 (100.00)	50 (100.00)
Anti-HCV/HCV RNA Positive	42	42 (100.00)	42 (100.00)
Increased Risk for HCV Infection[b]	25	8 (32.00)	8 (100.00)
Total	117	100 (85.47)	100 (100.00)

[a] A positive result was defined as reactive to two or more gene products by an immunoblot assay.

[b] Category included the following: intravenous drug users (5), hemophilia patients (10), men sex men (5), and female prostitutes (5).

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 34 panel set of seroconversion panels obtained from commercial vendors were tested on the Alinity i analyzer using the Alinity i Anti-HCV assay. The results were compared to a commercially-available anti-HCV assay and showed equivalent performance. Representative data from 5 panels are summarized in the following table.

Panel ID	Days Since 1st Bleed	Alinity i Anti-HCV Reactive ≥ 1.00 S/CO	Commercially-Available Anti-HCV Assay Reactive ≥ 1.00 S/CO
6214	0	0.15	0.10
	2	0.09	0.09
	8	0.07	0.08
	10	0.51	0.07
	16	0.38	0.08
	18	0.07	0.08
	23	0.36	0.38
	25	**1.14**	**1.36**
	32	**6.27**	**6.14**
	49	**11.31**	**12.08**
	53	**12.34**	**11.76**
	56	**12.39**	**12.17**
6229	0	0.30	0.29
	3	0.29	0.29
	7	0.27	0.26
	10	0.34	0.34
	17	**1.60**	**1.75**
	20	**2.54**	**2.55**
	24	**4.27**	**3.94**
	28	**8.40**	**8.60**
9047	0	0.05	0.04
	2	0.05	0.05
	10	0.07	0.06
	12	0.04	0.05
	19	0.05	0.05
	21	0.06	0.05
	28	**4.79**	**4.54**
	30	**9.36**	**9.73**
	35	**10.67**	**10.63**
	37	**11.40**	**10.76**
PHV914	0	0.08	0.08
	5	0.06	0.07
	9	0.07	0.07
	12	0.17	0.17
	16	**1.42**	**1.39**
	19	**1.72**	**1.81**
	24	**4.46**	**4.89**
	30	**6.85**	**7.30**
	33	**8.80**	**8.51**
PHV920	0	0.05	0.07
	5	0.08	0.10
	7	0.14	0.17
	13	**1.70**	**1.80**
	16	**5.77**	**6.03**
	20	**5.96**	**6.67**
	26	**8.28**	**8.61**
	28	**9.38**	**9.80**
	33	**11.52**	**11.89**
	35	**11.76**	**12.20**

Other Specimen Conditions or Disease States

This study was performed on the ARCHITECT i System.

A total of 104 specimens from individuals with other disease states or medical conditions unrelated to HCV infection were evaluated. Of the 104 specimens, 3 were repeatedly reactive and were anti-HCV positive by supplemental testing. The results demonstrated a specificity of 100.00%.

Category	Number Tested	IR (% of Total)	RR (% of Total)	Number of Positive by Supplemental Testing[a] (% of Repeatedly Reactive)
Other Specimen Conditions or Disease States [b]	104	3 (2.88)	3 (2.88)	3 (100.00)

IR = Initially Reactive; RR = Repeatedly Reactive

[a] A positive result was defined as reactive to two or more gene products by an immunoblot assay.

[b] The specimens included the following: anti-CMV positive (5), anti-EBV positive (5), anti-HAV positive (5), HBsAg positive (12), anti-HIV-1 positive (5), syphilis (5), rheumatoid factor (11), alcoholic liver disease (5), anti-HBc positive (5), anti-HTLV-I positive (10), anti-HTLV-II positive (2), human anti-mouse antibody positive (10), influenza vaccine recipients (5), pregnant women 1st trimester (7), *T. gondii* positive (5), and multiple myeloma (7).

Interference

This study was performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

No qualitative performance differences were observed between experimental controls and a minimum of 23 nonreactive or 23 spiked reactive specimens tested with elevated levels of the compounds listed in the table below.

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

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Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).

Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
INVERSIONS PERFORMED	Inversions Performed
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRODUCT OF GERMANY	Product of Germany
REF	List Number
SN	Serial number

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