Alinity s

Anti-HCV II Reagent Kit

en
Anti-HCV II
04W56
H14970R01
B4W5G0

Created July 2022.

REF 04W5660

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

NAME

Alinity s Anti-HCV II Reagent Kit

Hepatitis C Virus (*E coli*, Recombinant) NS3 Helicase Antigens and Synthetic Core Peptide

INTENDED USE

The Alinity s Anti-HCV II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum and plasma specimens on the Alinity s System.

The Alinity s Anti-HCV II assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HCV. The assay is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing serum and EDTA plasma specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

This test is not intended for use as an aid in diagnosis of infection with HCV.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV) is the causative agent of acute and chronic hepatitis infection. Globally, an estimated 58 million individuals are chronically infected. In 2019, approximately 290 000 people died from HCV-related liver disease, primarily due to cirrhosis and hepatocellular carcinoma (primary liver cancer).[1]

HCV belongs to the genus Hepacivirus in the family Flaviviridae and is a linear, single-stranded, positive-sense RNA virus. It is divided into at least 6 different genotypes (1-6) and several subtypes based on nucleotide sequence homology.[2] Each HCV genotype can be present in any given country, but there are geographical differences in prevalence. Differences between genotypes are associated with responses to treatment.[3]

HCV is spread through contact with blood from an infected person, such as sharing needles to inject drugs. Less commonly, HCV is transmitted through blood transfusion, sexual or perinatal routes or contact with contaminated personal items. Because of effective blood screening using serological and nucleic acid testing (NAT) methods, the risk of transfusion-transmitted HCV infections has been reduced.[4]

HCV RNA can be detected within a few days of exposure to HCV, prior to the development of antibodies.[2] This time period, referred to as the pre-seroconversion window period, often extends for several weeks after initial infection with HCV. In general, antibodies to HCV are absent in the early weeks of infection and are not detected on average until 8-11 weeks after infection.[5] In general 55%-85% of HCV infected individuals develop chronic infection, which is characterized by the continued detection of both HCV RNA and antibodies to HCV, persisting for decades after initial infection.[1, 2] About 30% of infected individuals resolve their infection, which is characterized by continued detection of antibodies to HCV, but with HCV RNA no longer being detectable.[1, 2]

Anti-HCV assays are used to identify individuals infected with HCV and to prevent transmission of the virus to recipients of blood or blood products. The Alinity s Anti-HCV II assay is designed to detect antibodies to recombinant antigens representing Core and NS3 regions of the HCV genome.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

Sample, streptavidin-coated microparticles precomplexed with biotinylated HCV constructs, acridinium-labeled HCV conjugate, and assay diluent are combined to create a reaction mixture and incubated. The anti-HCV present in the sample binds to the HCV-coated microparticles and to the acridinium-labeled HCV conjugate. The mixture is washed. Ancillary wash buffer is added and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLU). There is a direct relationship between the amount of anti-HCV in the sample and the RLU 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 s System Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity s Anti-HCV II Reagent Kit 04W56

NOTE: This product is composed of 4 components, which are packaged as a 2-cartridge reagent set. Both cartridges are required to perform the assay.

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

REF	04W5660
Tests per cartridge set	500
Number of cartridge sets per kit	5
Tests per kit	2500
MICROPARTICLES	27.0 mL
CONJUGATE	13.8 mL
ASSAY DILUENT	14.0 mL
ANCILLARY WASH BUFFER	26.5 mL

MICROPARTICLES Streptavidin-coated microparticles precomplexed with biotinylated HCV antigens (*E coli*, recombinant) and biotinylated HCV Core synthetic peptide in pyrophosphate-buffered saline with surfactants. Minimum concentration: 0.097% solids. Preservative: sodium azide.

CONJUGATE Acridinium-labeled HCV antigens (*E coli*, recombinant) and acridinium-labeled HCV Core synthetic peptide conjugate in pyrophosphate-buffered saline. Minimum concentration: Peptide 50 ng/mL, antigens 200 ng/mL. Preservative: sodium azide.

ASSAY DILUENT Pyrophosphate-buffered saline with surfactants. Preservative: sodium azide.

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REF	04W5660
ANCILLARY WASH BUFFER	Pyrophosphate-buffered saline. Preservative: sodium azide.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Performance characteristics of this product have not been established for laboratory diagnosis of HCV infection.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.[6-9]

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE / ASSAY DILUENT / ANCILLARY WASH BUFFER	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
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.transfusion.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity s System Operations Manual, Section 8.

Reagent Handling

- Do not invert reagent cartridges.
- Upon receipt, reagent cartridges can be used immediately or stored in an upright position.
- 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.
- 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 s System Operations Manual, Section 7.

Reagent Storage

- Do not freeze.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Opened	2 to 15°C	15 days after opening*	Store in upright position. Discard after 15 days. If cartridge does not remain upright during storage off the system, 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.

* Includes time on board the system.

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 15°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 s System 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 s System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity s Anti-HCV II Assay File must be installed on the Alinity s System prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity s System Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity s System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity s System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

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

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

Specimen Type	Anticoagulant
Serum (including serum separator tubes)	Not Applicable
Plasma	Dipotassium EDTA (including plasma preparation tubes)
	Tripotassium EDTA
	Lithium heparin (including plasma separator tubes)
	Sodium citrate
	Sodium heparin
	ACD-A
	ACD-B
	CP2D
	CPD
	CPDA-1

- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual specimens.
- Performance has not been established for the use of umbilical cord blood or bodily fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid.
- Performance has been established for the use of cadaveric serum and EDTA plasma specimens (including specimens collected postmortem, non-heart-beating) that have been collected up to 24 hours after death.[10]
- Testing of cadaveric serum and EDTA plasma 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 has not been verified.
- For cadaveric donors, serum and EDTA plasma may be used; follow general standards and/or regulations for collection, storage and handling.
- The system 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 with the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
  - specimens with obvious microbial contamination
  - specimens with fungal growth
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

Failure to follow the specified centrifugation procedure may give erroneous or inconsistent test results.

- Clear, nonhemolyzed specimens should be used when possible. Specimens containing visible particulate matter may give erroneous or inconsistent test results.
- 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.
- Prior to centrifugation, previously frozen specimens (including previously frozen plasmapheresis specimens) must be mixed gently and thoroughly after thawing.
- Specimens collected by plasmapheresis, which have not been frozen, do not require centrifugation. All other specimens (including previously frozen plasmapheresis specimens) must be centrifuged between 30 000 - 75 000 g-minutes.
- All specimens must be tested or retested within 48 hours of initial centrifugation. After 48 hours, these specimens need to be recentrifuged between 30 000 - 75 000 g-minutes.

The acceptable time and force ranges that meet this criterion are listed in the table below.

Centrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	3000	30 000
15	2000 - 3000	30 000 - 45 000
20	1500 - 3000	30 000 - 60 000
25	1300 - 3000	32 500 - 75 000

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

Convert RCF to rpm as follows:

$$rpm = 1000 \times \sqrt{\frac{RCF}{1.12 \times r_{max}}}$$

RCF - The relative centrifugal force generated during centrifugation.

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

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

r_{max} - Radius of the rotor in millimeters. The radius measured is dependent on whether the rotor is a fixed angle rotor or a swinging bucket rotor. This value is typically provided with the rotor by the manufacturer. For the fixed angle rotor, r_{max} is the measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor or rotor adapter. For the swinging bucket rotor, r_{max} is the measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor adapter or bucket at full extension.

NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) 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).

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Living Donor Serum/ Plasma	Room temperature (15 to 30°C)	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	14 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	-20°C or colder	9 months	Remove serum or plasma from the clot, red blood cells, or separator gel.

- Living donor specimens stored at -20°C or colder for greater than 9 months may be used for informational purposes (*e.g.*, lookback testing, discordant sample testing, clinical and validation testing).

- Storage at a combination of 15 to 30°C and 2 to 8°C may not exceed 14 days (inclusive of shipping time) and cannot exceed the maximum durations listed in the table above.
- Performance has not been established for living donor specimens that have undergone more than 6 freeze/thaw cycles.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Cadaveric Serum/ EDTA Plasma	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	14 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.
	-20°C or colder	9 months	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.

- Cadaveric specimens stored at -20°C or colder for greater than 9 months may be used for informational purposes (*e.g.*, lookback testing, discordant sample testing, clinical and validation testing).
- Storage at a combination of 15 to 30°C and 2 to 8°C may not exceed 14 days (inclusive of shipping time) and cannot exceed the maximum durations listed in the table above.
- Performance has not been established for cadaveric specimens that have undergone more than 6 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

04W56 Alinity s Anti-HCV II Reagent Kit

Materials Required but not Provided

- Alinity s Anti-HCV II Assay File
- 04W5603 Alinity s Anti-HCV II Calibrator Kit
- 04W5620 Alinity s Anti-HCV II Assay Control Kit
- 04W5624 Alinity s Anti-HCV II Release Control Kit
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity s Concentrated Wash Buffer

For information on materials required for operation of the system, refer to the Alinity s System Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity s System Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity s System Operations Manual, Section 5.

- Primary tubes may be on board the system for up to 10 hours.
- If using primary or aliquot tubes, refer to the Alinity s System 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
  - ≤ 3 hours on the reagent and sample manager:
    - Sample volume for first test: 350 µL
    - Sample volume for each additional test from same sample cup: 150 µL
  - > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity s Anti-HCV II Calibrator Kit, Assay Control Kit, and/or Release Control Kit package inserts for preparation and usage.
- For general operating procedures, refer to the Alinity s System Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity s System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Calibration

For instructions on performing a calibration, refer to the Alinity s System Operations Manual, Section 5.

Three replicates of Alinity s Anti-HCV II Calibrator 1 are automatically tested by the system. The calibrator must be priority loaded.

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

Once a calibration is accepted and stored, it may be used for 14 days. During this time, 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 quality control limits used to monitor and control system performance.

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

Quality Control Procedures

Assay Controls

The Alinity s Anti-HCV II Assay Controls must be tested once every 24 hours when the system is being used.

Assay control values must be within the ranges specified in the Alinity s Anti-HCV II Assay Control Kit package insert. When the assay control values are within range, sample results are generated, and a valid release control result is required to release test results. If an assay control value is not within range, sample results are not generated for in-process or scheduled samples. For troubleshooting information, refer to the Alinity s System Operations Manual, Section 10.

Release Controls

The Alinity s Anti-HCV II Release Control must be tested in order to release test results.

The release control is tested at user-defined intervals. For configuring the release control, refer to the Alinity s System Operations Manual, Section 2. For manually ordering the release control, refer to the Alinity s System Operations Manual, Section 5.

The release control must meet specifications defined in the Alinity s Anti-HCV II Release Control Kit package insert in order to validate the system functionality and release test results. If the release control does not meet specifications, refer to the Alinity s System Operations Manual, Section 10, for additional information.

Other Controls

Additional controls may be tested at operator's discretion in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy. For additional information on configuring customer controls, refer to the Alinity s System Operations Manual, Section 2.

Invalidate controls: Additional controls may be tested anywhere within a run as an invalidate control. Specifications may be assigned to invalidating controls. If an invalidate control fails to meet assigned specifications, no sample results are calculated or provided by the system. When an invalidate control meets assigned specifications, sample processing continues, and a valid release control result is required to release test results.

Non-validating controls: Additional controls may be tested anywhere within a run as a non-validating control. Specifications may be assigned to non-validating controls. A valid release control result is required to release test results. If the user-assigned specifications for the non-validating control(s) are not met and the release control specifications are met, there will be no effect on sample processing. In this case, reactive sample results must not be considered invalid.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[11]

RESULTS

Calculation

The Alinity s System calculates results for the Alinity s Anti-HCV II 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 0.219

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

Initial Result (S/CO)	Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required. Specimen considered negative for antibodies to HCV.
≥ 1.00	Reactive	Retest in duplicate.

Final Interpretation

Retest Results (S/CO)	Final Results	Final Interpretation
Both results < 1.00	Nonreactive	Specimen considered negative for antibodies to HCV.
One or both results ≥ 1.00	Repeatedly Reactive	Specimen should be further tested by supplemental methods.

Supplemental methods should follow appropriate FDA recommendations and regulations for specimens found to be repeatedly reactive.

Customers outside the US must follow their country's government recommendations and regulations for specimens found to be repeatedly reactive.

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 s System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- Potential interference has not been evaluated for substances other than those described in the **SPECIFIC PERFORMANCE CHARACTERISTICS - Interference** section of this package insert.
- False reactive results can be expected with any test kit. Falsely elevated results have been observed due to non-specific interactions (refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section of this package insert).
- Although the association of infectivity and the presence of antibodies to HCV is strong, it is recognized that presently available methods for antibodies to HCV detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HCV infection. A nonreactive test result does not exclude infection.

Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Reproducibility

A study was performed based on guidance from CLSI EP05-A3.[12] Testing was conducted using 3 lots of the Alinity s Anti-HCV II Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. Panel members and controls were tested twice a day for 5 days in replicates of 4 at 3 sites.

		Mean	Within-Run		Between-Run		Between-Day		Within-Laboratory[a]		Between-Site		Between-Lot		Reproducibility[b]	
Sample	N	S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	360	0.81	0.022	2.7	0.007	0.9	0.008	1.0	0.024	3.0	0.004	0.5	0.044	5.4	0.051	6.3
Low HCV Antibody	360	1.28	0.031	2.4	0.004	0.3	0.006	0.5	0.031	2.5	0.010	0.8	0.065	5.1	0.074	5.8
High HCV Antibody	360	11.12	0.282	2.5	0.000	0.0	0.000	0.0	0.282	2.5	0.000	0.0	0.534	4.8	0.610	5.5
Positive Control	360	2.82	0.053	1.9	0.000	0.0	0.000	0.0	0.053	1.9	0.000	0.0	0.072	2.6	0.093	3.3
Negative Control	360	0.05	0.003	NA	0.001	NA	0.001	NA	0.003	NA	0.001	NA	0.011	NA	0.012	NA

%CV = Coefficient of Variation expressed as a percentage; N = Number of Replicates; NA = Not Applicable: %CVs are not meaningful when S/CO approaches zero; SD = Standard Deviation

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

[b] Includes within-run, between-run, between-day, between-site, between-lot, and site-lot interaction variability

Specificity

A total of 5277 fresh serum specimens and 7082 fresh plasma specimens from volunteer whole blood donors were collected at 3 distinct blood centers. A total of 3167 specimens from plasmapheresis donors were collected at one additional blood center. Both the initial and repeat reactive rates for the serum specimens were 0.11% (6/5277), both the initial and repeat reactive rates for the plasma specimens were 0.11% (8/7082), and both the initial and repeat reactive rates for the plasmapheresis donor specimens were 0.19% (6/3167). Repeatedly reactive specimens were further tested using an HCV qualitative RNA assay and an FDA-approved immunoassay for anti-HCV. Based on supplemental test results for the repeatedly reactive specimens, 14 specimens were positive, and 6 specimens were negative.

Specificity based on assumed zero prevalence of antibody to HCV in whole blood and plasmapheresis donors was estimated in this study to be 99.96% (15 506/15 512) with a 95% confidence interval of 99.92% to 99.99%.

Specimen Category	Number Tested	IR (% of Total) (95% CI)	RR (% of Total) (95% CI)	Number Positive by Supplemental Testing (% of RR)	Specificity (%)[a] (95% CI)
Volunteer Blood Donors - Serum	5277	6 (0.11) (0.04 - 0.25)	6 (0.11) (0.04 - 0.25)	3 (50.00)	99.94 (5271/5274) (99.83 - 99.99)
Volunteer Blood Donors - Plasma	7082	8 (0.11) (0.05 - 0.22)	8 (0.11) (0.05 - 0.22)	6 (75.00)	99.97 (7074/7076) (99.90 - 100.00)
Total Volunteer Blood Donors	**12 359**	**14 (0.11) (0.06 - 0.19)**	**14 (0.11) (0.06 - 0.19)**	**9 (64.29)**	**99.96 (12 345/12 350) (99.91 - 99.99)**
Plasmapheresis Donors	3167	6 (0.19) (0.07 - 0.41)	6 (0.19) (0.07 - 0.41)	5 (83.33)	99.97 (3161/3162) (99.82 - 100.00)
Total Donors	**15 526**	**20 (0.13) (0.08 - 0.20)**	**20 (0.13) (0.08 - 0.20)**	**14 (70.00)**	**99.96 (15 506/15 512) (99.92 - 99.99)**

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

[a] Based on supplemental test results for the 20 repeatedly reactive specimens, 14 specimens were positive (3 blood donor serum, 6 blood donor plasma, and 5 plasmapheresis donor specimens), and 6 specimens were negative (3 blood donor serum, 2 blood donor plasma, and 1 plasmapheresis donor specimens); all 14 repeatedly reactive specimens found to be positive by supplemental testing were excluded from the specificity calculations.

For total donors, the IR rate not reactive on retest was estimated to be 0.00% (0/15 506) with a 95% confidence interval of 0.00% to 0.08%.

IR Rate Not Reactive on Retest = 100% × (Number of IR − Number of RR) / (Number Tested − Number of RR)

Sensitivity

A total of 807 specimens from the categories shown in the table below were tested using the Alinity s Anti-HCV II assay at 3 clinical sites. Repeatedly reactive specimens from individuals at increased risk of HCV infection were tested using an HCV qualitative RNA assay and an FDA-approved immunoassay for anti-HCV.

Sensitivity was estimated to be 100.00% (403/403) with a 95% confidence interval of 99.09% to 100.00% for preselected positive specimens.

Specimen Category	Number Tested	Number Positive	Number RR (% of Total)	Number RR Positive by Supplemental Testing (% of RR)	Sensitivity (%) (95% CI)
Preselected Anti-HCV Positive[a]	403[c]	403	403 (100.00)	403 (100.00)	100.00 (403/403) (99.09 - 100.00)
Individuals at Increased Risk of HCV Infection[b]	404	70	80 (19.80)	70 (87.50)	100.00 (70/70) (94.87 - 100.00)
Total	**807**	**473**	**483 (59.85)**	**473 (97.93)**	**100.00 (473/473) (99.22 - 100.00)**

CI = Confidence Interval; RR = Repeatedly Reactive

[a] Preselected anti-HCV positive specimens were positive by anti-HCV assays and an FDA-licensed HCV RNA nucleic acid test.

[b] The following risk factors were diagnosed or treated for HIV, sexual contact with HIV infected individual, hemodialysis patient, high risk sex behaviors, history of incarceration, illicit drug use (intravenous or intranasal), intranasal cocaine user, intravenous drug user, men who have sex with men, multiple sex partners, occupational exposure, tattoo, body piercing or acupuncture, persons with known exposure to HCV, and transfusion recipient (received transfusion prior to July 1992 or received transfusion from HCV positive donor).

[c] Three of the 403 preselected anti-HCV positive specimens were from individuals diagnosed with acute infection based on medical diagnosis and HCV RNA and/or anti-HCV results.

Genotype Detection

A total of 131 preselected HCV positive specimens of known genotype (genotypes 1-6) obtained from commercial vendors were tested using the Alinity s Anti-HCV II assay. The results were compared to a commercially available anti-HCV assay. All 131 specimens were repeatedly reactive using the Alinity s Anti-HCV II assay and the commercially available anti-HCV assay.

Seroconversion Sensitivity

The seroconversion detection of the Alinity s Anti-HCV II assay was compared to an FDA-licensed commercially available anti-HCV assay. Thirty-eight seroconversion panel sets, consisting of 310 total panel members, obtained from commercial vendors were tested using the Alinity s Anti-HCV II and commercially available anti-HCV assays. In 15 panels, the Alinity s Anti-HCV II and commercially available anti-HCV assays were reactive on the same bleed. In 10 panels, the 2 assays were reactive within 1 bleed. In 9 panels, the Alinity s Anti-HCV II assay was reactive 2 to 6 bleeds earlier than the commercially available anti-HCV assay, and in 4 panels, all bleeds were nonreactive with the commercially available anti-HCV assay while the Alinity s Anti-HCV II assay was reactive in the last bleed.

Other Specimen Conditions or Disease States

A total of 314 specimens from individuals with other disease states or medical conditions unrelated to HCV infection were evaluated. Of the 314 specimens, 1 was repeatedly reactive using the Alinity s Anti-HCV II assay and was anti-HCV negative by supplemental testing.

Specimen Category	Number Tested	IR (% of Total)	RR (% of Total)	Number Positive by Supplemental Testing (% of Repeatedly Reactive)
Other Specimen Conditions or Disease States[a]	314	1 (0.32)	1[b](0.32)	0 (0.00)

IR = Initially Reactive; RR = Repeatedly Reactive

[a] The specimens included the following: Anti-HIV-1/HIV-2 Positive (12), Anti-HTLV I/II Positive (11), HBV Positive (25), Anti-HAV Positive (15), Anti-HDV Positive (12), Anti-CMV (15), Co-infected CMV/EBV/HSV (14), Anti-*T pallidum* Positive (15), Non-viral Hepatitis (15), Rheumatoid Factor Positive (15), Anti-ds DNA Positive (11), Pregnant Females (15), Multiparous Females (15), Hyper IgG/IgM (11), Influenza Vaccine Recipient (15), Hemodialysis Patients (15), HAMA Positive (15), *E coli* Infection (13), Heterophilic Antibody Positive (14), Fungal (Yeast) Infection (15), ANA Positive (13), and Autoimmune Hepatitis (13).

[b] One HBV positive specimen was repeatedly reactive using the Alinity s Anti-HCV II assay and negative by supplemental testing.

Interference

Potentially Interfering Endogenous Substances

A study was performed testing potential interferents at or above the concentrations recommended in CLSI EP37.[13]

No interference was observed using the Alinity s Anti-HCV II assay from potentially interfering substances (spiked) at the levels shown below.

Potentially Interfering Substance	Interferent Level
Conjugated Bilirubin	≤ 40 mg/dL
Unconjugated Bilirubin	≤ 40 mg/dL
Hemoglobin	≤ 1000 mg/dL
Triglycerides	≤ 3000 mg/dL
Total Protein	≤ 15 g/dL

In addition, a negative and positive serum specimen were spiked with biotin to a concentration of 4250 ng/mL. No interference was observed using the Alinity s Anti-HCV II assay.

The effect of potentially interfering substances has only been evaluated for those listed in this package insert.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Twenty-three cadaveric serum specimens, 23 cadaveric EDTA plasma specimens, 23 living donor serum specimens, and 23 living donor plasma specimens were spiked with human plasma reactive for anti-HCV to create low-level reactive specimens. Each specimen was tested once per day over 6 days using each of 3 lots of the Alinity s Anti-HCV II Reagent Kit. Total %CV values were determined.

Specimen Category	Specimen Matrix	Number of Replicates	Mean S/CO	Total[a]	
				SD	%CV
Cadaveric	Serum[b]	414	3.41	0.125	3.7
	EDTA Plasma[c]	414	3.35	0.109	3.2
Living Donor	Serum	414	3.93	0.113	2.9
	EDTA Plasma	414	3.90	0.096	2.5

[a] Total variability contains within-specimen, between-lot and lot-specimen interaction variance components.

[b] Cadaveric serum specimens were collected up to 28.2 hours after death.

[c] Cadaveric EDTA plasma specimens were collected up to 39.8 hours after death.

Specificity

Specificity was determined by testing 55 cadaveric serum specimens, 55 cadaveric EDTA plasma specimens, 55 living donor serum specimens, and 55 living donor plasma specimens. Each specimen was tested once using each of 3 lots of the Alinity s Anti-HCV II Reagent Kit.

Specimen Category	Specimen Matrix	Lot	Nonreactive	Repeatedly Reactive	Specificity (%) (95% CI)
Cadaveric	Serum[a]	1	55	0	100.00 (93.51 - 100.00)
		2	55	0	100.00 (93.51 - 100.00)
		3	55	0	100.00 (93.51 - 100.00)
	EDTA Plasma[b]	1	55	0	100.00 (93.51 - 100.00)
		2	55	0	100.00 (93.51 - 100.00)
		3	55	0	100.00 (93.51 - 100.00)
Living Donor	Serum	1	55	0	100.00 (93.51 - 100.00)
		2	55	0	100.00 (93.51 - 100.00)
		3	55	0	100.00 (93.51 - 100.00)
	Plasma	1	55	0	100.00 (93.51 - 100.00)
		2	55	0	100.00 (93.51 - 100.00)
		3	55	0	100.00 (93.51 - 100.00)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 28.2 hours after death.

[b] Cadaveric EDTA plasma specimens were collected up to 39.8 hours after death.

Analytical Sensitivity

Cadaveric serum and EDTA plasma specimens and living donor serum and plasma specimens were spiked with human plasma reactive for anti-HCV to create low-level and high-level reactive specimens. Each specimen was tested once, within 24 hours of spiking, using each of 3 lots of the Alinity s Anti-HCV II Reagent Kit. All specimens were reactive on all 3 reagent lots.

Specimen Category	Analyte Level	Specimen Matrix	Lot	Number of Specimens	Mean S/CO	Sensitivity (%) (95% CI)
Cadaveric	Low Positive	Serum[a]	1	55	3.69	100.00 (93.51 - 100.00)
			2	55	3.63	100.00 (93.51 - 100.00)
			3	55	3.69	100.00 (93.51 - 100.00)
		EDTA Plasma[b]	1	55	3.66	100.00 (93.51 - 100.00)
			2	55	3.67	100.00 (93.51 - 100.00)
			3	55	3.69	100.00 (93.51 - 100.00)
	High Positive	Serum[a]	1	55	9.27	100.00 (93.51 - 100.00)
			2	55	8.97	100.00 (93.51 - 100.00)
			3	55	9.26	100.00 (93.51 - 100.00)
		EDTA Plasma[b]	1	55	9.14	100.00 (93.51 - 100.00)
			2	55	8.97	100.00 (93.51 - 100.00)
			3	55	9.09	100.00 (93.51 - 100.00)
Living Donor	Low Positive	Serum	1	55	4.31	100.00 (93.51 - 100.00)
			2	55	4.18	100.00 (93.51 - 100.00)
			3	55	4.32	100.00 (93.51 - 100.00)
		Plasma	1	55	4.09	100.00 (93.51 - 100.00)
			2	55	4.00	100.00 (93.51 - 100.00)
			3	55	4.10	100.00 (93.51 - 100.00)
	High Positive	Serum	1	55	10.89	100.00 (93.51 - 100.00)
			2	55	10.43	100.00 (93.51 - 100.00)
			3	55	10.86	100.00 (93.51 - 100.00)
		Plasma	1	55	10.60	100.00 (93.51 - 100.00)
			2	55	10.08	100.00 (93.51 - 100.00)
			3	55	10.54	100.00 (93.51 - 100.00)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 28.2 hours after death.

[b] Cadaveric EDTA plasma specimens were collected up to 39.8 hours after death.

Cadaveric Specimen Storage

Cadaveric specimen storage was determined by testing a minimum of 10 low-level reactive cadaveric serum specimens, 10 low-level reactive cadaveric EDTA plasma specimens, 10 nonreactive cadaveric serum specimens, and 10 nonreactive cadaveric EDTA plasma specimens. The low-level reactive specimens were prepared by spiking 10 nonreactive cadaveric serum specimens and 10 nonreactive cadaveric EDTA plasma specimens to a target S/CO value near the cutoff with human plasma reactive for anti-HCV. Each specimen was tested at Day 0, and then subjected to either 2 to 8°C storage for 14 days, room temperature (15 to 30°C) storage for 3 days, −20°C or colder storage for 12 months, or 6 freeze/thaw cycles. Nonreactive specimens were evaluated by calculating the differences between the mean S/CO of Day 0 and the mean S/CO of each storage condition and related timepoint. Reactive specimens were evaluated by calculating the percent differences between the mean S/CO of Day 0 and the mean S/CO of each storage condition and related timepoint. There were no changes to the interpretation; the data demonstrate that cadaveric serum and EDTA plasma specimens can be stored at the following conditions when tested using the Alinity s Anti-HCV II assay.

			Nonreactive Specimens	Reactive Specimens
Storage Condition	Timepoint	Specimen Matrix	Upper Limit of 2-sided 95% CI of Differences	Lower Limit of 2-sided 95% CI of % Differences
Room Temperature (15 to 30°C)	3 days	Serum[a,b]	-0.01 S/CO	-10.6%
		EDTA Plasma[c,d]	-0.01 S/CO	-9.0%
2 to 8°C	14 days	Serum[a,b]	0.00 S/CO	-8.6%
		EDTA Plasma[c,d]	0.00 S/CO	-9.2%
-20°C or colder	12 months	Serum[a,b]	0.00 S/CO	6.2%
		EDTA Plasma[c,d]	0.02 S/CO	5.4%
Freeze/Thaw	6 cycles	Serum[a,b]	0.01 S/CO	0.1%
		EDTA Plasma[c,d]	0.01 S/CO	1.1%

CI = Confidence Interval

[a] Hemoglobin levels of cadaveric serum specimens ranged from 37 to 902 mg/dL.

[b] Cadaveric serum specimens were collected up to 25.5 hours after death.

[c] Hemoglobin levels of cadaveric EDTA plasma specimens ranged from 10 to 210 mg/dL.

[d] Cadaveric EDTA plasma specimens were collected up to 24.5 hours after death.

Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS**, Specimen Storage section of this package insert for the maximum storage times allowed.

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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
ANCILLARY WASH BUFFER	Ancillary Wash Buffer
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
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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