Anti-HBc Reagent Kit

Hepatitis B Virus Core Antigen (*E coli*, Recombinant)

Revised July 2019

REF 06P0660

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

■NAME

Alinity s Anti-HBc Reagent Kit

Hepatitis B Virus Core Antigen (*E coli*, Recombinant)

■INTENDED USE

The Alinity s Anti-HBc assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma specimens on the Alinity s System.

The Alinity s Anti-HBc 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-HBc. 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 specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

■ SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is the causative agent of hepatitis B. An estimated 257 million individuals are living with hepatitis B virus infection. More than 887 000 people die annually of HBV-related liver disease. Globally, chronic hepatitis B is a major cause of liver cirrhosis and hepatocellular carcinoma.[1, 2]

HBV belongs to the hepadnavirus family and is a partially double stranded DNA virus. It consists of a central core nucleocapsid containing the viral DNA, DNA polymerase, and a surrounding envelope consisting of hepatitis B surface antigen (HBsAg), which is expressed during HBV infection. Additionally, HBV-infected cells produce spherical or long filamentous particles that consist of excess HBsAg.[3]

The virus is divided into multiple major serotypes (e.g., adr, adw, ayr, ayw) based on antigenic determinants present on the envelope proteins, and into at least 8 genotypes (A–H) according to overall nucleotide sequence variation of the genome. Differences among genotypes can affect the disease severity, course and likelihood of complications, response to treatment, and possibly vaccine protection.[2-5]

HBV is transmitted through sexual, parenteral, and perinatal routes. Transmission may also occur through transfusion of HBV-contaminated blood and blood products. After infection with HBV, antibody to the hepatitis B core antigen (anti-HBc) appears in the serum one to two weeks after the appearance of HBsAg. Because it generally remains detectable for the remainder of a patient's life, anti-HBc is an indicator of current infection (acute or chronic) or of past infection.[3, 6]

Anti-HBc assays are used to screen blood and blood products for the presence of anti-HBc to prevent transmission of HBV infection to recipients of blood or blood products. Anti-HBc assays are also used to screen organ and tissue donors. In addition, anti-HBc assays are used in the diagnosis of HBV infection in combination with other hepatitis B serological markers.[3, 7-10]

■BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

Sample, recombinant hepatitis B core antigen (rHBcAg) coated paramagnetic microparticles, assay diluent, and specimen diluent are combined and incubated. The anti-HBc present in the sample binds to the rHBcAg coated microparticles. The mixture is washed. Anti-human IgG and 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 (RLU). There is a direct relationship between the amount of anti-HBc in the sample and the RLU detected by the system optics. The presence or absence of anti-HBc 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-HBc Reagent Kit 06P06

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	06P0660
Tests per cartridge set	500
Number of cartridge sets per kit	5
Tests per kit	2500
MICROPARTICLES	27.0 mL
CONJUGATE	29.0 mL
ASSAY DILUENT	24.2 mL
SPECIMEN DILUENT	24.0 mL

MICROPARTICLES Hepatitis B core (*E coli*, recombinant) antigen coated microparticles in TRIS buffer and surfactant. Minimum concentration: 0.08% solids. Preservatives: ProClin 950, sodium azide.

CONJUGATE Murine anti-human IgG and IgM acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizers and surfactant. Minimum concentration: (IgG) 23 ng/mL, (IgM) 17 ng/mL, Preservatives: sodium alkyl paraben, sodium azide.

ASSAY DILUENT Protein (mouse) stabilizers and surfactant in MOPSO buffer. Preservatives: ProClin 950, sodium azide.

SPECIMEN DILUENT Reductant in MOPSO buffer.

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Warnings and Precautions

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

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.[11-14]

The following warnings and precautions apply to: MICROPARTICLES	
	
WARNING	Contains methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: ASSAY DILUENT	
	
DANGER	Contains polyethylene glycol octylphenyl ether (Triton X-405), methylisothiazolone and sodium azide.
H318	Causes serious eye damage.
H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
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.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

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.

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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-HBc 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 Types	Anticoagulants
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 plasmapheresis 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 specimens (including specimens collected post-mortem, non-heart-beating) that have been collected up to 24 hours after death.[15] Follow general standards and/or regulations for collection, storage and handling.
- Performance has not been established for the use of cadaveric plasma specimens.
- Testing of cadaveric serum 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.
- 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 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 must be mixed gently and thoroughly after thawing.
- All 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	3 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 3 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	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	3 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.

- Performance has not been established using cadaveric specimens stored at −20°C or colder for greater than 3 months.
- 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

06P06 Alinity s Anti-HBc Reagent Kit

Materials Required but not Provided

- Alinity s Anti-HBc Assay File
- 06P0603 Alinity s Anti-HBc Calibrator Kit
- 06P0620 Alinity s Anti-HBc Assay Control Kit
- 06P0624 Alinity s Anti-HBc 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: 225 µL
    - Sample volume for each additional test from same sample cup: 25 µL
  - > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity s Anti-HBc 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-HBc 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-HBc 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-HBc 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-HBc 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-HBc 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.[16]

■RESULTS

Calculation

The Alinity s System calculates results for the Alinity s Anti-HBc 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 1.00

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 HBc.
≥ 1.00	Reactive	Retest in duplicate.

Final Interpretation

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

Additional methods should follow appropriate FDA recommendations and regulations for specimens found to be repeatedly reactive. Customers outside the U.S. 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 may be 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 HBc is strong, it is recognized that presently available methods for HBc antibody detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HBV 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 EP15-A2.[17] Testing was conducted using 3 lots of the Alinity s Anti-HBc 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.

			Within-Run		Between-Run		Between-Day		Within- Laboratory[a]		Between-Site		Between-Lot		Reproducibility[b]	
Sample	N	Mean S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low HBc Antibody	360	1.96	0.043	2.2	0.016	0.8	0.017	0.9	0.049	2.5	0.009	0.5	0.129	6.6	0.144	7.4
High HBc Antibody	360	9.93	0.222	2.2	0.000	0.0	0.079	0.8	0.235	2.4	0.051	0.5	1.339	13.5	1.374	13.8
Positive Control	360	2.86	0.063	2.2	0.015	0.5	0.032	1.1	0.072	2.5	0.000	0.0	0.118	4.1	0.148	5.2
Negative Control	360	0.10	0.006	NA	0.002	NA	0.000	NA	0.006	NA	0.004	NA	0.010	NA	0.013	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 the site-lot interaction variability.

Specificity

A total of 9365 fresh serum specimens and 6512 fresh plasma specimens from volunteer whole blood donors were collected at 3 distinct blood centers. The initial and repeat reactive rates for the serum specimens were 0.21% (20/9365) and 0.21% (20/9365), respectively. The initial and repeat reactive rates for the plasma specimens were 0.37% (24/6512) and 0.37% (24/6512), respectively. Repeatedly reactive specimens were further tested for one or more additional markers: HBV qualitative DNA, HBsAg, anti-HBc IgM, anti-HBs, and anti-HBe. Based on supplemental test results, 28 specimens were positive and 16 specimens were negative.

Specificity based on assumed zero prevalence of antibody to HBc in whole blood donors was estimated in this study to be 99.90% (15 833/15 849) with a 95% confidence interval of 99.84% to 99.94%.

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	9365	20 (0.21) (0.13 – 0.33)	20 (0.21) (0.13 – 0.33)	9 (45.00)	99.88 (9345 / 9356) (99.79 – 99.94)
Volunteer Blood Donors- Plasma	6512	24 (0.37) (0.24 – 0.55)	24 (0.37) (0.24 – 0.55)	19 (79.17)	99.92 (6488 / 6493) (99.82 – 99.97)
Total Donors	**15 877**	**44 (0.28) (0.20 – 0.37)**	**44 (0.28) (0.20 – 0.37)**	**28 (63.64)**	**99.90 (15 833 / 15 849) (99.84 – 99.94)**

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

[a] Based on supplemental test results for the 44 repeatedly reactive specimens, 28 specimens were positive (9 blood donor serum and 19 blood donor plasma), and 16 specimens were negative (11 blood donor serum and 5 blood donor plasma). All 28 repeatedly reactive specimens found to be positive by supplemental testing were excluded from the specificity calculations.

For total donors, IR rate not reactive on retest was estimated to be 0.00% (0/15 833) with a 95% confidence interval of 0.00% to 0.02%.

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-HBc assay at 3 clinical sites. Repeatedly reactive specimens from individuals at increased risk of HBV infection were further tested for one or more additional markers: HBV DNA, HBsAg, anti-HBc IgM, anti-HBs, and anti-HBe. Sensitivity was estimated to be 100.00% (404/404) with a 95% confidence interval of 99.09% to 100.00% for preselected positive specimens.

			Alinity s Anti-HBc		
Specimen Category	Number Tested	Number Positive	Number RR (% of Total)	Number RR that were Positive (% of RR)	Sensitivity (%) (95% CI)
Preselected Anti-HBc Positive - Acute HBV Infection[a]	28	28	28 (100.00)	28 (100.00)	100.00 (28/28) (87.66 - 100.00)
Preselected Anti-HBc Positive - Chronic HBV Infection[a]	97	97	97 (100.00)	97 (100.00)	100.00 (97/97) (96.27 - 100.00)
Preselected Anti-HBc Positive - Recovered HBV Infection[a]	279	279	279 (100.00)	279 (100.00)	100.00 (279/279) (98.69 - 100.00)
Subtotal	**404**	**404**	**404 (100.00)**	**404 (100.00)**	**100.00 (404/404) (99.09 - 100.00)**
Increased Risk of HBV Infection[b]	403	70	84[c] (20.84)	70 (83.33)	100.00 (70/70) (94.87 - 100.00)
Total	**807**	**474**	**488 (60.47)**	**474 (97.13)**	**100.00 (474/474) (99.22 - 100.00)**

RR = Repeatedly Reactive; CI = Confidence Interval

[a] Preselected anti-HBc positive specimens were previously identified as reactive by two FDA approved anti-HBc assays. Acute, chronic and recovered HBV classifications were determined using four HBV reference markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs).

[b] The following risk factors were included: current or past residence in a Hepatitis B endemic region, diagnosed or treated for a sexually transmitted disease, hemodialysis patient, heterosexual contact with a high-risk individual or an infected individual, history of incarceration, household contact with HBV infected individual, intravenous drug user, men who have sex with men, and multiple sex partners.

[c] Of the 14 specimens that were repeatedly reactive and negative by supplemental testing, 13 were also repeatedly reactive with a commercially available anti-HBc assay.

Analytical Sensitivity

Analytical sensitivity was evaluated using dilutions of the WHO 1st International Standard for anti-hepatitis B core antigen (anti-HBc), NIBSC code: 95/522. The dilutions ranged from 0.25 to 2.50 IU/mL. The dilutions were tested across 3 lots of the Alinity s Anti-HBc Reagent Kit on 1 Alinity s System. The analytical sensitivity of the Alinity s Anti-HBc assay ranged from 0.53 IU/mL to 0.56 IU/mL.

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Seroconversion Sensitivity

To determine the seroconversion sensitivity, 10 seroconversion panels obtained from commercial vendors were tested on the Alinity s System using the Alinity s Anti-HBc assay. The results were compared to a commercially available anti-HBc assay and representative data from 5 panels are summarized in the following table.

Panel ID	Days Since 1st Bleed	Alinity s Anti-HBc Reactive ≥ 1.00 S/CO	Commercially-Available Anti-HBc Assay Reactive ≤ 1.00 S/CO
13867/3482[a]	27	0.03	1.82
	29	0.03	1.72
	34	0.03	1.74
	36	0.05	1.66
	41	**1.30**	**0.96**
	43	**3.28**	**0.64**
	63	**9.09**	**0.17**
	70	**8.68**	**0.20**
	72	**8.71**	**0.19**
1807/3463[b]	41	0.06	1.72
	44	0.06	1.74
	49	0.07	1.58
	63	**2.53**	**0.41**
	64	**3.03**	**0.39**
	69	**3.11**	**0.41**
	71	**3.47**	**0.36**
	76	**6.48**	**0.17**
43527/3453[c]	0	0.09	1.67
	9	0.09	1.66
	13	0.21	1.42
	34	**1.29**	1.20
	37	**1.93**	1.05
	41	**3.88**	**0.65**
	44	**4.31**	**0.51**
	48	**3.90**	**0.59**
26982/14399[d]	10	0.06	1.63
	15	0.06	1.63
	18	0.06	1.60
	24	**3.09**	**0.48**
	27	**4.23**	**0.34**
	34	**4.88**	**0.41**
	38	**4.79**	**0.46**
	41	**4.70**	**0.42**
HBV6281[e]	19	0.05	1.83
	22	0.07	1.92
	33	0.06	1.77
	36	0.04	1.69
	41	**1.55**	**0.92**
	43	**4.06**	**0.42**
	50	**5.92**	**0.35**
	54	**6.02**	**0.40**

[a] Seven early bleeds are not shown as they are all nonreactive. Fifteen subsequent bleeds are not shown as they are all reactive.
[b] Eight early bleeds are not shown as they are all nonreactive. Nine subsequent bleeds are not shown as they are all reactive.
[c] Eighteen subsequent bleeds are not shown as they are all reactive.
[d] Three early bleeds are not shown as they are all nonreactive. Fourteen subsequent bleeds are not shown as they are all reactive.
[e] Four early bleeds are not shown as they are all nonreactive.

Other Specimen Conditions or Disease States

A total of 207 specimens from individuals with other specimen conditions or disease states unrelated to HBV infection were evaluated. Of the 207 specimens, 31 were repeatedly reactive using the Alinity s Anti-HBc assay.

Thirty of the 31 specimens were repeatedly reactive using a commercially available anti-HBc assay and 29 specimens were positive 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]	207	31 (14.98)	31 (14.98)	29[b,c] (93.55)

IR = Initially Reactive; RR = Repeatedly Reactive

[a] The specimens included the following: Anti-HIV-1/HIV-2 Positive (10), Anti-HTLV I/II Positive (10), Anti-HCV Positive (10), Anti-HAV Positive (10), Co-infected CMV/EBV/HSV (10), Anti-*T pallidum* Positive (10), Non-Viral Hepatitis Positive (10), Rheumatoid Factor Positive (10), Anti-ds DNA Positive (10), Pregnant Females (14), Multiparous Females (10), Hyper IgG/IgM (9), Influenza Vaccine Recipients (10), Hemodialysis Patients (10), HAMA Positive (10), *E coli* Infection (10), Heterophilic Antibody Positive (25), Fungal (Yeast) Infection (10), and Anti-HDV Positive (9).
[b] One anti-HCV positive specimen and 1 heterophilic antibody positive specimen were repeatedly reactive using the Alinity s Anti-HBc assay and negative by supplemental testing.
[c] Three anti-HCV positive specimens, 3 anti-HAV positive specimens, 1 co-infected CMV/EBV/HSV specimen, 2 anti-*T pallidum* positive specimens, 3 hyper IgG/IgM specimens, 1 HAMA positive specimen, 2 *E coli* infection specimens, 6 heterophilic antibody positive specimens, and 8 anti-HDV positive specimens were positive by supplemental testing.

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.[18]

No interference was observed using the Alinity s Anti-HBc assay from potentially interfering substances at the levels shown below.

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

In addition, a negative and positive control were spiked with biotin to a concentration of 4250 ng/mL. No interference was observed using the Alinity s Anti-HBc 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 donor serum specimens and 22 living donor serum specimens were spiked with human plasma reactive for anti-HBc to create low level reactive specimens.

Each specimen was tested once per day for 6 days using each of 3 lots of the Alinity s Anti-HBc Reagent Kit. Total %CV values were determined.

			Total[a]	
Specimen Category	Number of Replicates	Mean S/CO	SD	%CV
Cadaveric[b]	414	3.63	0.334	9.2
Living Donor	396	3.62	0.324	9.0

[a] Total variability contains within-specimen, between-lot and lot-specimen interaction variance components.
[b] Cadaveric serum specimens were collected up to 14.6 hours after death.

Specificity

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

Specimen Category	Lot	Nonreactive	Repeatedly Reactive	Specificity (%) (95% CI)
Cadaveric[a]	Lot 1	53	2[b]	100.00 (93.28 – 100.00)
	Lot 2	53	2[b]	100.00 (93.28 – 100.00)
	Lot 3	53	2[b]	100.00 (93.28 – 100.00)
Living Donor	Lot 1	52	3[c]	96.30 (87.25 – 99.55)
	Lot 2	52	3[c]	96.30 (87.25 – 99.55)
	Lot 3	52	3[c]	96.30 (87.25 – 99.55)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 23.7 hours after death.

[b] Two cadaveric donor specimens were positive per supplemental testing; therefore, they were excluded from the specificity calculation.

[c] One living donor specimen was positive per supplemental testing; therefore, it was excluded from the specificity calculation. Two additional living donor specimens were repeatedly reactive by the investigational method but had insufficient sample volume to complete all supplemental test methods; therefore, the results were classified as false positive and included in the specificity calculation.

Analytical Sensitivity

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

Specimen Category	Lot	Number of Specimens	Mean S/CO	Sensitivity (%) (95% CI)
Cadaveric[a]	Lot 1	52	3.76	100.00 (93.15 – 100.00)
	Lot 2	52	3.97	100.00 (93.15 – 100.00)
	Lot 3	52	4.48	100.00 (93.15 – 100.00)
Living Donor	Lot 1	52	3.74	100.00 (93.15 – 100.00)
	Lot 2	52	4.00	100.00 (93.15 – 100.00)
	Lot 3	52	4.44	100.00 (93.15 – 100.00)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 23.7 hours after death.

Cadaveric Specimen Storage

Cadaveric specimen storage was determined by testing a minimum of 12 low-level reactive specimens, prepared by spiking nonreactive cadaveric serum specimens to a target S/CO value near the cutoff with human plasma reactive for anti-HBc, and a minimum of 12 nonreactive cadaveric serum specimens. 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 3 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 specimens can be stored at the following conditions when tested using the Alinity s Anti-HBc assay.

Storage Condition	Timepoint	Nonreactive Specimens: Upper Limit of 2-sided 95% CI of Differences	Reactive Specimens: Lower Limit of 2-sided 95% CI of % Differences
Room Temperature (15 to 30°C)[a,c]	3 days	0.00 S/CO	–4.1%
2 to 8°C [a,c]	14 days	0.01 S/CO	–4.8%
–20°C or colder[b,d]	3 months	0.01 S/CO	0.2%
Freeze/Thaw [a,c]	6 cycles	0.00 S/CO	–13.3%

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 10.0 hours after death.

[b] Cadaveric serum specimens were collected up to 14.5 hours after death.

[c] Hemoglobin levels of cadaveric serum specimens ranged from 34 to 255 mg/dL.

[d] Hemoglobin levels of cadaveric serum specimens ranged from 45 to 2229 mg/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
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
SPECIMEN DILUENT	Specimen Diluent

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