Alinity s

HBsAg Confirmatory Reagent Kit

Antibody to Hepatitis B Surface Antigen (Sheep)

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HBsAg Conf
06P03
G92057R01
B6P0W0

Created June 2019.

REF 06P0359

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

NAME

Alinity s HBsAg Confirmatory Reagent Kit (also referred to as HBsAg Conf)

Antibody to Hepatitis B Surface Antigen (Sheep)

INTENDED USE

The Alinity s HBsAg Confirmatory assay is used to confirm the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma by means of specific antibody neutralization on the Alinity s System. The assay is intended to be used for confirmation of samples found to be repeatedly reactive by the Alinity s HBsAg assay.

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 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, unlike other DNA viruses, replicates through a reverse transcription step. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate more than 10 times higher than the mutation rate of other DNA viruses. Surface antigen gene mutations may cause changes in the antigenic structure of HBsAg, resulting in reduced recognition by some antibodies to HBsAg.[6-11]

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, HBsAg is the first antigenic marker, appearing 1 to 12 weeks after exposure and 2 to 6 weeks before the onset of clinical symptoms. HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within 6 months indicates a chronic hepatitis B infection.[2, 3]

HBsAg assays are used to screen blood and blood products for the presence of HBsAg to prevent transmission of HBV infection to recipients of blood or blood products. HBsAg assays are also used to screen organ and tissue donors. In addition, HBsAg assays are used to identify persons infected with HBV and to monitor the status of infected individuals in combination with other hepatitis B serological markers. Testing for HBsAg as part of an antenatal screening program may identify HBV infected mothers and allow for appropriate immunoprophylaxis of the newborn. Specific antibody neutralization assays are used to confirm the presence of HBsAg.[12-18]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay uses the Alinity s HBsAg assay reagents in addition to the reagents described in the **REAGENTS** section of this package insert. For information on the Alinity s HBsAg assay, refer to the Alinity s HBsAg Reagent Kit package insert.

This assay is used to confirm the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma, using chemiluminescent microparticle immunoassay (CMIA) technology. This assay consists of two single tests that are both pre-treatment immunoassays.

Sample and Pre-Treatment 1 are combined and incubated. When HBsAg is present in the sample, it is neutralized by the antibody (anti-HBs) in Pre-Treatment 1. The pretreated sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture and incubated. Any non-neutralized HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. The neutralized HBsAg is blocked from forming a sandwich with anti-HBs coated microparticles and acridinium-labeled anti-HBs conjugate. The mixture is washed. Ancillary wash buffer is added and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. This sequence is repeated for the sample and Pre-Treatment 2, except Pre-Treatment 2 does not contain anti-HBs and will not neutralize HBsAg in the sample.

The resulting chemiluminescent reaction is measured as relative light units (RLU). There is a direct relationship between the amount of HBsAg in the sample and the RLU detected by the system optics.

The Alinity s HBsAg Confirmatory sample to cutoff (S/CO) result is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

If the non-neutralized sample (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff of 0.70 S/CO and the RLU of the neutralized sample (incubated with Pre-Treatment 1) is reduced by at least 50% compared to the non-neutralized sample, the sample is considered confirmed positive for HBsAg.

For additional information on system and assay technology, refer to the Alinity s System Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity s HBsAg Confirmatory Reagent Kit 06P03

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

REF	06P0359
Tests per cartridge	42
Number of cartridges per kit	1
Tests per kit	42
PRE-TREATMENT 1	4.2 mL
PRE-TREATMENT 2	23.3 mL

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REF	06P0359
PRE-TREATMENT 1	Recalcified sheep plasma reactive for anti-HBs and recalcified human plasma. Preservatives: ProClin 950 and sodium azide.
PRE-TREATMENT 2	Recalcified human plasma and recalcified sheep plasma. Preservatives: ProClin 950 and sodium azide.

For each specimen to be tested, the Alinity s System performs an undiluted interpretation. The system performs automated dilution(s) of the sample if the assay interpretation is inconclusive. The system performs automated retest of the sample if the assay interpretation is invalid. The Alinity s HBsAg Confirmatory Reagent Kit supports up to 42 undiluted interpretations, or a combination of undiluted and diluted interpretations.

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 contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be 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 human sourced material used in Pre-Treatment 1 and Pre-Treatment 2 is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.

The following warnings and precautions apply to: PRE-TREATMENT 1 and PRE-TREATMENT 2

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.

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	30 days after opening*	Store in upright position. Discard after 30 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 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 HBsAg and HBsAg Confirmatory Assay Files 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.

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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 umbilical cord blood or bodily fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid.
- Performance has not been established for the use of cadaveric blood specimens with the Alinity s HBsAg Confirmatory assay.
- 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
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.

- 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 donor specimens that have undergone more than 6 freeze/thaw cycles.
- 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).

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

06P03 Alinity s HBsAg Confirmatory Reagent Kit

Materials Required but not Provided

- Alinity s HBsAg Assay File
- Alinity s HBsAg Confirmatory Assay File
- 06P02 Alinity s HBsAg Reagent Kit
- 06P0204 Alinity s HBsAg Calibrator Kit
- 06P0213 Alinity s HBsAg Assay Control Kit
- 06P0215 Alinity s HBsAg 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: 472 µL*
    - Sample volume for each additional test from same sample cup: 272 µL*
  - > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity s HBsAg 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.

* Sample volume is based on sufficient quantity required to perform all dilutions, if necessary.

Calibration

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

The Alinity s HBsAg Confirmatory assay uses a calibration generated with the Alinity s HBsAg assay and Alinity s HBsAg Calibrator Kit.

Three replicates of Alinity s HBsAg Calibrator 1 and Calibrator 2 are automatically tested by the system. The calibrators must be priority loaded. The calibration must be evaluated using both the Alinity s HBsAg and Alinity s HBsAg Confirmatory assays before Alinity s HBsAg Confirmatory assay results can be generated. The Alinity s HBsAg Negative Control and Alinity s HBsAg Positive Control must be tested with the Alinity s HBsAg assay, and the Alinity s HBsAg Positive Control must also be tested with the Alinity s HBsAg Confirmatory assay.

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 HBsAg Negative Control and Alinity s HBsAg Positive Control must be tested with the Alinity s HBsAg assay, and the Alinity s HBsAg Positive Control must be tested with the Alinity s HBsAg Confirmatory assay at least once every 24 hours when the system is being used.

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

Release Controls

The Alinity s HBsAg Release Control must be tested in order to release test results.

The release control is tested at user-defined intervals using the Alinity s HBsAg assay. 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 the specifications defined in the Alinity s HBsAg 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.[23]

RESULTS

Calculation

The Alinity s System calculates results for the Alinity s HBsAg Confirmatory 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 0.0575) + (Calibrator 2 mean RLU x 0.8)] x 0.75

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

The Alinity s System calculates the % Neutralization result for the Alinity s HBsAg Confirmatory assay using the Pre-Treatment 1 (HBsAgCf C2) and Pre-Treatment 2 (HBsAgCf C1) results for each specimen and control using the following equation:

% Neutralization = [(Sample with HBsAgCf C1 RLU) - (Sample with HBsAgCf C2 RLU)] / [(Sample with HBsAgCf C1 RLU) - (Calibrator 2 Mean RLU)] × 100

The result is based on S/CO and % Neutralization of the sample. If the sample's HBsAgCf C1 S/CO is <0.70, % neutralization is not applicable. Obtain the final interpretation of results directly from the table in **Interpretation of Results** section in this package insert.

Interpretation of Results

Undiluted

HBsAgCf C1 (S/CO)	% Neutralization	Interpretation
< 0.70	Not Applicable	Not Confirmed
≥ 0.70	< -15%	Invalid Sample is automatically retested with no dilution.
≥ 0.70 to < 10.00	≥ -15% to < 50%	Not Confirmed
≥ 0.70	≥ 50%	Confirmed Positive
≥ 10.00	≥ -15% to < 50%	Inconclusive Sample is automatically retested with 1:500 dilution.

1:500 Dilution

HBsAgCf C1 (S/CO)	% Neutralization	Interpretation
< 0.70	Not Applicable	Not Confirmed
≥ 0.70	< -15%	Invalid Sample is automatically retested with 1:500 dilution.
≥ 0.70	≥ 50%	Confirmed Positive
≥ 0.70	≥ -15% to < 50%	Inconclusive Sample is automatically retested with 1:18 750 dilution.

1:18 750 Dilution

HBsAgCf C1 (S/CO)	% Neutralization	Interpretation
< 0.70	Not Applicable	Not Confirmed
≥ 0.70	< -15%	Invalid Sample is automatically retested with 1:18 750 dilution.
≥ 0.70	≥ 50%	Confirmed Positive
≥ 0.70	≥ -15% to < 50%	Not Confirmed

- Follow the dilution and final interpretation routine as outlined in the table above, even if % neutralization results > 100% are obtained.
- The interpretation of Not Confirmed for HBsAg indicates that the presence of HBsAg cannot be confirmed through neutralization. The repeatedly reactive result obtained with the Alinity s HBsAg assay may be the result of a nonspecific reaction (false reactive). As the presence of nonspecific binding may obscure low levels of HBsAg in the specimen due to early infection or early recovery, a Not Confirmed result does not exclude HBV infection.

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.
- Although the association of infectivity and the presence of HBsAg is strong, it is recognized that presently available methods for HBsAg detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HBV infection.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[24] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed.
- Vaccination with a recombinant HBsAg hepatitis B vaccine may cause transient positive results caused by a passive transfer of antigen by vaccination.

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.[25] Testing was conducted using 3 lots of the Alinity s HBsAg Reagent Kit, HBsAg Confirmatory Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. Panel members and positive control were tested twice a day for 5 days in replicates of 4 at 3 sites.

			Within-Run		Between-Run		Between-Day		Within-Laboratory[b]		Between-Site		Between-Lot		Reproducibility[c]	
Sample	**N**	**Mean S/CO[a]**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low HBsAg	360	1.75	0.081	4.6	0.015	0.9	0.028	1.6	0.087	4.9	0.000	0.0	0.038	2.2	0.098	5.6
High HBsAg	360	8.40	0.345	4.1	0.091	1.1	0.000	0.0	0.357	4.2	0.000	0.0	0.120	1.4	0.400	4.8
Positive Control	360	2.55	0.098	3.8	0.000	0.0	0.028	1.1	0.102	4.0	0.000	0.0	0.024	0.9	0.112	4.4

			Within-Run		Between-Run		Between-Day		Within-Laboratory[b]		Between-Site		Between-Lot		Reproducibility[c]	
Sample	**N**	**Mean %Neut**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low HBsAg	360	94.98	3.913	4.1	0.705	0.7	0.673	0.7	4.032	4.2	1.421	1.5	3.822	4.0	6.275	6.6
High HBsAg	360	99.05	0.663	0.7	0.257	0.3	0.000	0.0	0.711	0.7	0.205	0.2	0.672	0.7	1.115	1.1
Positive Control	360	97.37	2.337	2.4	0.374	0.4	0.000	0.0	2.366	2.4	0.928	1.0	2.376	2.4	3.808	3.9

%CV = Coefficient of Variation expressed as a percentage; N = Number of Replicates; %Neut = % Neutralization; SD = Standard Deviation

[a] Pre-Treatment 2 S/CO

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

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

Confirmation of HBsAg Reactive Specimens

Preselected Positive and Increased Risk specimens that were repeatedly reactive by the Alinity s HBsAg assay were evaluated using the Alinity s HBsAg Confirmatory assay.

Specimen Category	**N**	**Number of Alinity s HBsAg RR (% of Total)**	**Number Confirmed Positive[a] (% of RR) (95% CI)**
Preselected HBsAg Positive[b]	167	167 (100.00)	167 (100.00) (97.82 - 100.00)
Preselected HBsAg Positive – Acute HBV Infection[b]	70	70 (100.00)	70 (100.00) (94.87 - 100.00)
Preselected HBsAg Positive – Chronic HBV Infection[b]	195	195 (100.00)	195 (100.00) (98.13 - 100.00)
Total Preselected Positive	**432**	**432 (100.00)**	**432 (100.00) (99.15 - 100.00)**
Increased Risk of HBV Infection[c]	403	3 (0.74)	3 (100.00) (NA)[d]
HBsAg Positive Genotypes A-H	16	16 (100.00)	16 (100.00) (79.4 - 100.00)

NA = Not Applicable; RR = Repeatedly Reactive; CI = Confidence Interval

[a] A specimen was considered as confirmed positive if the result for the non-neutralized specimen (incubated with Alinity s HBsAg Confirmatory Pre-Treatment 2) was ≥ 0.70 S/CO and if neutralization with anti-HBs (Alinity s HBsAg Confirmatory Pre-Treatment 1) was ≥ 50%.

[b] Preselected HBsAg positive specimens were previously confirmed positive by specific antibody neutralization using FDA approved assays. Acute and chronic HBV classifications were determined using four HBV reference markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs) or by medical diagnosis.

[c] 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.

[d] The confidence interval is not meaningful due to the small number of specimens.

Presumed negative specimens from the following categories were evaluated using the Alinity s HBsAg assay. Specimens that were repeatedly reactive by the Alinity s HBsAg assay were evaluated using the Alinity s HBsAg Confirmatory assay.

Specimen Category	**N**	**Number of Alinity s HBsAg RR (% of Total)**	**Number Confirmed Positive[a] (% of RR)**
Volunteer Blood Donors - Serum	7347	5 (0.07)	2 (40.00)
Volunteer Blood Donors - Plasma	6511	3 (0.05)	0 (0.00)
Total Volunteer Blood Donors	**13 858**	**8 (0.06)**	**2 (25.00)[b]**
Plasmapheresis Donors	3135	0 (0.00)	NA
Total Donors	**16 993**	**8 (0.05)**	**2 (25.00)[b]**
Other Specimen Conditions or Disease States[c]	191	9 (4.71)	9 (100.00)[d]

NA = Not Applicable; RR = Repeatedly Reactive

[a] A specimen was considered as confirmed positive if the result for the non-neutralized specimen (incubated with Alinity s HBsAg Confirmatory Pre-Treatment 2) was ≥ 0.70 S/CO and if neutralization with anti-HBs (Alinity s HBsAg Confirmatory Pre-Treatment 1) was ≥ 50%.

[b] The 6 repeatedly reactive specimens that were not confirmed by the Alinity s HBsAg Confirmatory assay were nonreactive by a commercially available HBsAg assay.

[c] 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), Anti-HDV Positive (9), Co-infected CMV/EBV/HSV (10), Anti-*T pallidum* Positive (10), Non-viral Hepatitis (10), Rheumatoid Factor Positive (10), Anti-ds DNA Positive (10), Pregnant Females (14), Multiparous Females (10), Hyper IgG/IgM (10), Influenza Vaccine Recipient (10), Hemodialysis Patients (10), HAMA Positive (10), *E coli* Infection (9), Heterophilic Antibody Positive (9), and Fungal (Yeast) Infection (10).

[d] One anti-HIV-1/HIV-2 Positive and 8 anti-HDV Positive specimens were confirmed positive.

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.[26]

No interference was observed using the Alinity s HBsAg Confirmatory 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 positive control was spiked with biotin to a concentration of 4250 ng/mL. No interference was observed using the Alinity s HBsAg Confirmatory assay.

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

HBsAg Mutant Detection

A total of 52 preselected HBsAg positive mutant specimens (14 native mutant specimens and 38 recombinant mutant specimens) obtained from commercial vendors were tested using the Alinity s HBsAg Confirmatory assay. The results were compared to a commercially available HBsAg confirmatory assay.

Mutant	Alinity s HBsAg Confirmatory Interpretation	Commercially Available HBsAg Confirmatory Assay Interpretation
Native Mutant Specimens		
Ser-143-Leu + Pro-211-His	**CP**	**CP**
Gly-145-Ala + Thr-189-Ile	**CP**	**CP**
Thr-27-Lys + Tyr-100-Cys + Gln-129-Arg + Leu-175-Ser + Trp-199-Leu	**CP**	**CP**
Leu-49-Arg + Gln-101-His + Thr-126-Ile + Glu-164-Gly	**CP**	**CP**
Gly-145-Ala	**CP**	**CP**
Cys-76-Trp + Pro-120-Ser + Ser-132-Phe	**CP**	**CP**
Asp-144-Glu + Ser-204-Asn + Ser-207-Asn	**CP**	**CP**
Pro-127-Leu + Gln-129-His	**CP**	**CP**
Ser-143-Leu + Thr-189-Ile	**CP**	**CP**
Gly-145-Ala + Thr-189-Ile + Phe-212-Tyr	**CP**	**CP**
Ser-143-Leu	**CP**	**CP**
Thr-118-Lys + Thr-140-Ile + Cys-149-Tyr	**CP**	RRNC
Pro-135-Leu + Cys-139-Tyr + Asp-144-Ala + Gly-145-Arg + Ser-171-Tyr + Val-180-Ala	**CP**	RRNC
Phe-93-Cys + Met-103-Ile + Gly-145-Arg + Ser-174-Asn	**CP**	RRNC
Recombinant Mutant Specimens		
Cys-137-Tyr	**CP**	**CP**
Cys-147-Ser	**CP**	**CP**
Cys-124-Arg	**CP**	NA
122-Asp-Thr	**CP**	NA
Pro-120-Ser + Thr-125-Met + Pro-127-Tyr + Ser-143-Leu	**CP**	**CP**
Cys-121-Tyr + Lys-122-Leu + Thr-123-Asn + Gly-130-Glu + Met-133-Ile + Asp-144-Gly + Gly-145-Arg	**CP**	NA
Gln-129-His	**CP**	**CP**
Met-133-Leu	**CP**	**CP**
Asp-144-Ala	**CP**	**CP**
Gly-145-Arg	**CP**	**CP**
Pro-142-Leu + Gly-145-Arg	**CP**	**CP**
Pro-142-Ser + Gly-145-Arg	**CP**	**CP**
Thr-123-Ala	**CP**	NA
122-Asn-Thr	**CP**	NA
122-Arg-Ala	**CP**	NA
Thr-123-Asn	**CP**	NA
Gly-145-Lys	**CP**	**CP**
Thr-143-Leu	**CP**	**CP**
Thr-123-Ser	**CP**	NA
123-Arg-Gly-Ala	**CP**	NA
Thr-123-Ala + Gly-145-Arg	**CP**	NA
Gly-145-Glu	**CP**	**CP**
Met-133-Leu + Gly-145-Arg	**CP**	**CP**
Thr-126-Ser	**CP**	**CP**
Met-133-Thr	**CP**	**CP**
Gly-145-Ala	**CP**	**CP**
Pro-120-Ser + Asp-144-Glu + Gly-145-Arg + Thr-189-Ile	**CP**	**CP**
Phe-134-His + Pro-142-Leu + Asp-144-Glu + Gly-145-Arg	**CP**	**CP**
Thr-126-Ile	**CP**	**CP**
Thr-123-Asn + Thr-143-Ser	**CP**	NA
Thr-126-Ala + Met-133-Ile	**CP**	**CP**
Pro-127-Thr + Gly-145-Arg	**CP**	**CP**
Asp-144-Glu + Gly-145-Arg	**CP**	**CP**
Thr-126-Ile + Phe-134-His + Pro-142-Leu + Gly-145-Arg	**CP**	**CP**
Thr-143-Leu + Val-190-Ala + Tyr-200-Cys + Tyr-206-Arg	**CP**	**CP**
Leu-109-Ile + Gly-112-Lys + Ser-113-Ala + Pro-120-Thr + Phe-134-Ser	**CP**	**CP**
Ile-110-Arg + Lys-122-Tyr + Phe-134-Ser + Pro-142-Leu + Asp-144-Ala	**CP**	**CP**
Thr-125-Met + Thr-126-Asn + Pro-127-Thr	**CP**	**CP**

CP = Confirmed Positive; RRNC = Repeatedly Reactive Not Confirmed

NA = Not Applicable; specimen was nonreactive on screening assay

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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

	Caution
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
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INFORMATION FOR USA ONLY	Information needed for United States of America only
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