Anti-HBs

Antibody to Hepatitis B Surface Antigen (CLIA)

Order Information

Catalog No.	Package Size
Anti-HBs111	2×50 tests
Anti-HBs112	2×100 tests

Intended Use

The CL-series Anti-HBs assay is a Chemiluminescent Immunoassay (CLIA) for the quantitative determination of antibody to hepatitis B surface antigen (Anti-HBs) in human serum or plasma.

Summary

After the infection of Hepatitis B virus (HBV), Hepatitis B surface antigen (HBsAg) is the first serological marker to appear in blood. The increase in antibody to HBsAg (anti-HBs) accompanied with decrease in HBsAg might indicate convalescence or recovery from HBV infection. Anti-HBs assay is often used to monitor the clinical status of Hepatitis B infected individuals. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV. [1]

Hepatitis B vaccine can stimulate immune system to produce anti-HBs for preventing HBV infection.[1]Anti-HBs assay is commonly used to monitor the efficacy of Hepatitis B vaccination. The presence of anti-HBs is important for protection against Hepatitis B virus (HBV) infection.[2]

Antibody to HBsAg is mainly generated against the HBsAg determinant "a". HBsAg is rarely detected with Anti-HBs at the same time, except for HBV immune escape mutants during chronic infection.[3]

Assay Principles

The CL-series Anti-HBs assay is a two-site sandwich assay to determine the level of antibody to hepatitis B surface antigen (Anti-HBs).

In the first step, sample, paramagnetic microparticles coated with hepatitis B surface antigen (HBsAg), and alkaline phosphatase (ALP) labeled HBsAg are added into a reaction vessel. After incubation, antibody to hepatitis B surface antigen (Anti-HBs) in sample will bind to HBsAg coated on microparticles and HBsAg labeled with ALP to form sandwich complex (Microparticles -HBsAg-Anti-HBs-(HBsAg-ALP). Afterwards, the reaction vessel will be placed under the magnetic field. microparticles are magnetically captured while other unbound substances are removed by washing.

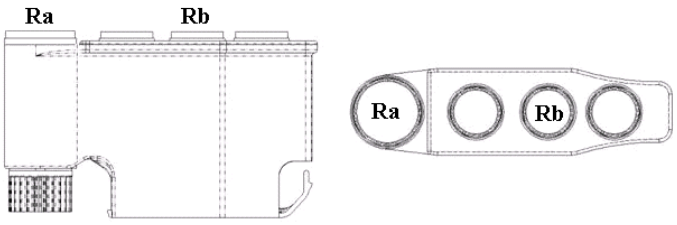
In the second step, the substrate solution is added to the reaction vessel. It is catalyzed by ALP in the immune complex captured on the microparticles. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by a photomultiplier built into the system. The amount of Anti-HBs present in the sample is proportional to the relative light units (RLUs) generated during the reaction. The Anti-HBs concentration can be determined via a calibration curve, which is built on an encoded Master Calibration Curve and three level product calibrators.

Reagent Components

The reagent kit is composed of two components: Ra and Rb. The component cannot be exchanged, and the detailed information of each component is listed below:

Ra	Paramagnetic microparticles coated with hepatitis B surface antigen in TRIS buffer containing preservative.
Rb	Alkaline phosphatase labeled hepatitis B surface antigen in diluent containing preservative.

The position of each component is shown in the figure below (front view on the left and top view on the right):



Storage Stability

The unopened Anti-HBs (CLIA) reagent kit is stable up to the expiration date as indicated on the label when stored at 2-8°C. The actual expiration date is stated on the label.

The Anti-HBs (CLIA) reagent kit can be stored onboard at 2-8°C, and used for a maximum of 28 days after opening for use.

Reagent Preparation

Ra: Ready to use

Rb: Ready to use

Materials required but not provided

Mindray CL-series Chemiluminescence Immunoassay Analyzer.

Cat. No. Anti-HBs 211: Anti-HBs Calibrators, 1×2.0mL for each level of calibrator C0, C1 and C2;

Cat. No. Anti-HBsN 311: Anti-HBs Negative Control 3×2.0mL;

Cat. No. Anti-HBsN 312: Anti-HBs Negative Control, 6×2.0mL;

Cat. No. Anti-HBsP 311: Anti-HBs Positive Control, 3×2.0mL;

Cat. No. Anti-HBsP 312: Anti-HBs Positive Control, 6×2.0mL;

Cat. No. CS511: Substrate Solution, 4×115mL;

Cat. No. WB411: Wash Buffer, 1x10 L;

Reaction Vessel

Instrument System

Mindray CL-series Chemiluminescence Immunoassay Analyzer.

Specimen Collection and Preparation

Human serum is recommended for this assay. Centrifuge the specimen at 3500 rpm for a minimum of 10 minutes after clot formation is complete. Specimen should be tested as soon as possible after sample collection and pre-analytical treatment. Test the specimen within two hours after centrifugation. If testing is not completed within 8 hours, transfer the supernatant into tubes for storage. Specimen should be tightly capped and refrigerated at 2-8°C. If testing will be delayed for more than 14 days, specimens should be frozen at -20°C or below.

Avoid repeated freeze and thaw cycles, which may cause sample deterioration. Specimen can be used after a maximum of five cycles of freeze and thaw.

Do not use specimen with the following conditions:

- heat inactivated
- grossly hemolyzed
- obvious microbial contamination
- visible fibrin or other cell debris

Assay Procedure

For optimal performance of this assay, operators should read the related system operation manual carefully to get sufficient information, such as operation instructions, sample preservation and management, safety precautions, and maintenance. Prepare all required materials for the assay as well.

Before loading the Anti-HBs reagent kit on the instrument for the first time, invert unopened reagent bottle gently for at least 30 times to resuspend the microparticles, which have settled during shipment or storage. Visually inspect the bottle to ensure the microparticles have been homogenized. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely mixed. If the microparticles cannot be homogenized, it is recommended not to use this bottle of reagent. Contact Mindray Customer Service for help. Do not invert opened reagent bottle.

This assay requires 50 µL of sample volume for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration

CL-series Anti-HBs has been calibrated against WHO 2nd International Standard anti-HBs antibody (NIBSC Code: 07/164).

The specific information of master calibration curve of Anti-HBs reagent kit is stored in the two-dimensional barcode attached in the reagent pack. It is used together with product calibrators for the calibration of the specific reagent lot. When performing the calibration, scan the information of master calibration curve from the barcode into the system first, and then use the three level product calibrators. Valid calibration curve is required before any Anti-HBs test. Recalibration is recommended every 4 weeks, or when a new reagent lot is used, or the quality controls are out of specified ranges. For detailed instruction of calibration, refer to the system operation manual.

Quality Control

It is recommended that quality controls should be run once every 24 hours if the tests are in use, or after every calibration. The quality control frequency should be adapted to each laboratory's individual requirements. The recommended two levels of quality controls for this assay are Mindray Anti-HBs Negative Control and Anti-HBs Positive Control.

Quality control results should be within the acceptable

ranges. If a control is out of its specified range, the associated test results are invalid and the samples must system operation manual to check the assay system. If the quality control results are still out of the specified range, please contact Mindray Customer Service for help.

Calculation

The analyzer automatically calculates the analyte concentration of each sample on the basis of master calibration curve read from the barcode, and a 4-Parameter Logistic Curve Fitting (4PLC) with the relative light units (RLUs) generated from three level product calibrators of defined concentrations. The results are shown in the unit of mIU/mL.

Dilution

Samples with Anti-HBs concentrations above the upper limit can be diluted with Mindray Sample Diluent. The recommended dilution is 1:40 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 10 IU/mL. After manual dilution, multiply the result by the dilution factor. After automated dilution by the analyzers, the system automatically multiply the result by the dilution factor when calculating the sample concentration.

Reference Range

Two kinds of recognized Anti-HBs assay kits were used to select 414 Anti-HBs non-reactive samples and 314 Anti-HBs reactive samples. These samples were tested by Anti-HBs kit on Mindray CL-2000i system. Data were analyzed with statistical analysis software (ROC curve). The specificity and sensitivity can vary according to selected Cutoff value. A Cutoff of10 mIU/mL with the best balanced specificity and sensitivity were determined as the Cutoff of Mindray Anti-HBs kit.

Samples tested with HBsAg kit on Mindray CL2000i system with a concentration less than cutoff (10 mIU/mL) is considered as Anti-HBs non-reactive; with a concentration larger than or equal to cutoff, is considered as Anti-HBs reactive.

Interpretation of Results

1. Samples with anti-HBs concentration of <10 mIU/mL are Non-reactive. These samples are considered as negative for anti-HBs, and do not need further testing.
2. Samples with anti-HBs concentration of ≥10 mIU/mL are Initially Reactive.
3. Initially Reactive samples with anti-HBs concentration between 10 and 100 mIU/mL need to be retested. Samples should be transferred to a centrifuge tube and centrifuged at≥10,000 RCF (Relative Centrifugal Force) for 10 minutes, and retested in duplicate.

Initially Reactive samples with anti-HBs concentration of <10 mIU/mL in both retests are negative for anti-HBs.

Initially Reactive samples with anti-HBs concentration of >10 mIU/mL in either of the retests are Repeatedly Positive for anti-HBs.

Repeatedly reactive samples as well as Initially Reactive samples with a concentration ≥100 mIU/mL must be confirmed according to the recommended confirmatory algorithms.

Limitation of Measurement

The upper limit of this assay is 1000 mIU/mL. A specimen with an anti-HBs concentration lower than the upper limit can be quantitatively determined, while specimen with a concentration higher than the upper limit will be reported as >1000 mIU/mL or diluting the samples with Mindray Sample Diluent.

The concentration of anti-HBs in a given specimen can vary, depending on manufacturers' measurement systems. It is due to the differences in assay methods, calibration, and reagent specificity. The assay results should be used in conjunction with other data, such as symptoms, results of other tests, and clinical history, etc to make clinical decisions.

Performance Characteristics

Sensitivity

The Anti-HBs (CLIA) reagent kit has an analytical sensitivity of ≤2.0 mIU/mL. Analytical sensitivity is defined as the lowest concentration of analyte that can be differentiated from a sample with no analyte. It is measured as the HBsAg concentration at two standard deviations above the mean RLU from 20 measurements of an analyte-free sample.

314 specimens have been claimed to be anti-HBs positive by reference methods. Mindray Anti-HBs assay on CL-2000i system has detected 308 samples as positive and 6 samples as negative. The positive coincidence rate is 98.10%.

Reportable Range

Reportable range is defined by the analytical sensitivity and the upper limit of the master calibration curve. The reportable range of Anti-HBs (CLIA) reagent kit is 2.0~1000.0 mIU/mL (or the upper limit is up to 40000 mIU /mL for 40-fold diluted samples).

Precision

The CL-series Anti-HBs assay is designed to have a precision of ≤10% (within-device CV). Precision was determined by following National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A2[4]. Two levels of quality controls were tested in duplicate in two separate runs per day, for a total of 20 days, using a single lot of reagents and a single calibration curve. The precision data are summarized in the table below.

Sample	Mean Anti-HBs (mIU/mL)	Within-Run CV	Between-Run CV	Within-Device CV
Negative Control	8.22	2.70%	2.15%	5.08%
Positive Control	257.09	1.97%	3.94%	5.65%

Specificity

Hemoglobin up to 500 mg/dL, bilirubin up to 20 mg/dL, triglycerides up to 1500 mg/dL, and total protein up to 10 g/dL will not interfere with the CL-series Anti-HBs assay. These substances show less than 10% interference at indicated concentrations.

Mindray Anti-HBs kit was evaluated for potential cross-reactive substances from individuals with medical conditions unrelated to HBV infection. At least 5 samples containing each of the potentially interfering pathogens of the following (HAV, HCV, HEV, HGV, HIV-1/2, TP, EBV, RV, CMV, HSV-1, Toxo Rubella, *E.coli*, Yeast, and lentivirus) are tested by Mindray Anti-HBs and a reference method. All results are consistent on both Mindray and the reference measurement procedure.

414 specimens have been claimed Anti-HBs negative by reference methods. Mindray Anti-HBs assay on CL-2000i system has detected 407 samples as negative and 7 samples as positive. The negative coincidence rate is 98.30%.

Hook Effect

There is no high dose hook effect at an Anti-HBs concentration up to 70, 000 mIU/mL.

Accuracy

Two trueness controls with defined values traceable to the International Standard were used to verify the accuracy of this assay. The results showed that the relative deviation was less than ±10%. The results are listed in the following table.

Sample	Measured Anti-HBs (mIU/mL)	Defined Anti-HBs (mIU/mL)	Relative Deviation
Level 1	21.16	20.36	3.94%
Level 2	150.91	146.8	2.80%

Linearity

A high concentration Anti-HBs sample (approximately 250 mIU/mL) was mixed with a low concentration sample (<5 mIU/mL) at different ratios, generating a series of dilutions. The total Anti-HBs of each dilution was determined using the Mindray CL-Series Anti-HBs Assay. Linearity was demonstrated in the range of 5.00 mIU/mL to 200.00 mIU/mL, the correlation coefficient r is ≥ 0.9900. The linearity data are summarized in the table below.

Anti-HBs (mIU/mL)	1	2	3	4	5	6
Expected	1.59	53.59	106.04	164.03	219.80	276.12
Measured	1.59	55.22	110.45	165.67	220.90	276.12

Method Comparison

The Mindray CL-Series Anti-HBs Assay was compared to a commercially available diagnostic kit in a correlation study. The statistical data obtained by Deming computing mode are shown in the table below.

Concentration Range (mIU/mL)	Slope	Intercept	Correlation Coefficient
0~1000 mIU/mL	1.45	1.90	0.949

Warnings and Precautions

1. For *in vitro* diagnostic use only.
2. Follow all the rules in handling laboratory reagents and take necessary safety precautions.
3. Due to the differences in methodology and antibody specificity, test results of the same sample may be different when using reagent kits from different manufacturers on Mindray system, or using Mindray reagent kits on other systems.
4. Do not use reagent kits beyond the expiration date.
5. Do not use reagents mixed from different reagent

lots.

6. Always keep the reagent pack in the upright position to ensure no microparticle has been lost prior to use.

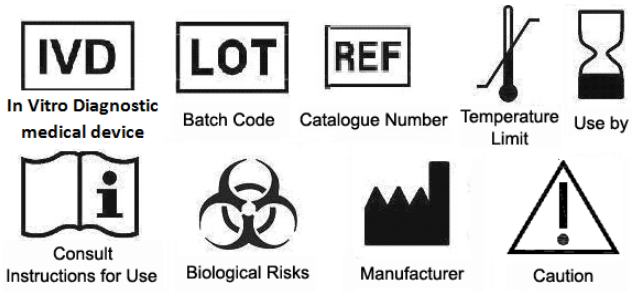
7. Reagent pack opened for more than 28 days is not recommended for use.

8. Reliability of assay results cannot be guaranteed if the instructions in this package insert are not followed.

9. All the specimen and reaction wastes should be considered potentially biohazard. Specimens and reaction wastes should be handled in compliance with the local regulations and guidelines.

10. The Material Safety Data Sheet (MSDS) is available upon request.

Graphical Symbols



Bibliography

1. Ambrosch F, Frisch-Niggemeyer W, Kremsner P, et al. Persistence of vaccine-induced antibodies to hepatitis B surface antigen and the need for booster vaccination in adult subjects. *Postgrad Med J* 1987; 63(S2):129-135.
2. Jilg W, Schmidt M, Deinhardt F. Immune response to hepatitis B revaccination. *J Med Virol* 1988;24: 377-384.
3. Lada O, Benhamou Y, Poynard T, *et al*. Coexistence of hepatitis B surface antigen (Anti-HBs) and anti-HBs antibodies in chronic hepatitis B virus carriers: influence of "a" determinant variants. *J Virol* 2006; 80: 2968-2975.
4. CLSI. EP5-A2: Vol. 24, No. 25, *Evaluation of Precision Performance of Quantitative Measurement Method*; Approved Guideline –Second Edition.

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