IVD

ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (ANTI-HBS)

CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0311-2

INTENDED USE
The AUTOBIO antibody to hepatitis B surface antigen (Anti-HBs) chemiluminescence immunoassay (CLIA) is intended for the quantitative determination of Anti-HBs concentration in human serum and plasma.

INTRODUCTION
Anti-HBs titer can be determined to monitor the prognosis of patients recovering from the hepatitis B viral infection. It also can be used as an indicator of prior exposure to Hepatitis B viruses. However, it is an immune response index for people who received HBV vaccine. [1-2]

The antibody response to hepatitis B virus surface antigen (anti-HBs) is an important serological marker for vaccine induced immunity to hepatitis B virus (HBV). An adequate vaccine response is defined as an anti-HBs level of higher than 100IU/l 4 to 8 weeks after the last of three or four vaccine injections. It is widely accepted that a sustained level of at least 10IU/l is protective against HBV infection. Vaccinees without sufficient anti-HBs responses, so-called nonresponders or low responders, undergo a special regimen of additional vaccine doses. For liver transplant recipients, quantitative measurement of anti-HBs levels is used in the management of hepatitis B immune globulin prophylaxis, which is initiated to maintain anti-HBs levels of at least 100IU/l or 200IU/l, according to different guidelines.[4-6]

These are divided into four categories based on Anti-HBs concentration
(Ⅰ) nonresponders: less than 1mIU/ml;
(Ⅱ) low responders: 1-10mIU/ml;
(Ⅲ)Medium responders: 10-100mIU/ml;
(Ⅳ)High responders: more than 100mIU/ml.

PRINCIPLE OF THE TEST
The Anti-HBs CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one HBsAg for solid phase (microtiter wells) immobilization and another HBsAg as antigen-enzyme (horseradish peroxidase) conjugate reagent. The Anti-HBs present in the reference standards and serum or plasma are "sandwiched" between the two antigens. Following the formation of the coated antigen-antibody-antigen-enzyme complex, the unbound antigen-enzyme labels are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The Related Light Unit (RLU) of the reaction is proportional to the concentration of Anti-HBs present in the specimen.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate coated with Hepatitis B Surface Antigen (HBsAg) (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled Hepatitis B Surface Antigen (HBsAg) in Stabilizing Buffer (1 vial, 6.0 ml)
3. Reference Standards:0(liguid.1vial,0.5ml),5(lyophilized)25(lyophilized),90(lyophilized), 300(lyophilized) and 1000(lyophilized)mIU/ml Anti-HBs in Stabilizing Buffer (6 vials,)
4. Substrate A: (1 vial,3.5 ml)
5. Substrate B: (1 vial, 3.5 ml)
6. PBS-T Powder: PBS-Tween (2 bags, 5g/ea)

MATERIALS NOT PROVIDED
The following materials are required but not provided in the kit.
1. Distilled water
2. Precision pipettes for delivery of 20-200 µl, 100-1000 µl (the use of accurate pipettes with disposable

    plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION
1. Unopened test kits should be stored at 2 ~ 8 ℃ upon receipt. The test kit may be used before the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted reference standards should be used within 30 days and be frozen at -20 ℃ for long term storage. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION
1. Human serum (including serum collected in serum separatortubes) or plasma collected in tubes containing potassium EDTA, lithium heparin,sodium heparin, sodium citrate and potassium oxalate may be used in the AUTOBIO Anti-HBs assay. Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient samples.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use，specimens should be capped and stored up to 48 hours at 2 ~ 8 ℃. For longer storage, freeze the specimens at -20 ℃. Thawed samples must be mixed prior to testing. Multiple freeze-thaw cycles should be avoided.

PRECAUTIONS AND WARNINGS
1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference standards contain a dilution of human plasma known to be positive for anti-HBs, which have been tested and found negative for antibody to HCV, HIV1 and HIV2. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the reference standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION
1. All reagents should be brought to room temperature (18 ~ 25 ℃) prior to use.
2. Reconstitute each lyophilized reference standard with 0.5 ml distilled water and the reference standards of 0mIU/ml can be used directly. Allow the reconstituted material to stand for at least 5 minutes. Reconstituted reference standards should be stored sealed at 2 ~ 8 ℃.
3. To prepare wash buffer: add 1 bag of PBS-T Powder to 500ml of distilled water, and mix well. The wash buffer is stable at room temperature at least for two weeks.

IMPORTANT NOTES
1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 48 wells be used for each assay run, if manual pipette is used, since pipetting of all reference standards, specimens and controls should be completed within 10 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50μl of reference standards, specimens, and controls into appropriate wells.
3. Dispense 50μl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 60 seconds. It is important to have complete mixing in this step.
5. Incubate at 37 ℃ for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and empty the microtiter plate 6 times with wash buffer either manually or with an automatic washer.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 25μl of Subsrate A, then25μl of Substrate B into each well. Gently mix for 10 seconds.
10. Put the microtiter plate into the detecting chamber of a Luminometer for 10 minutes, then read the RLU values of each well.
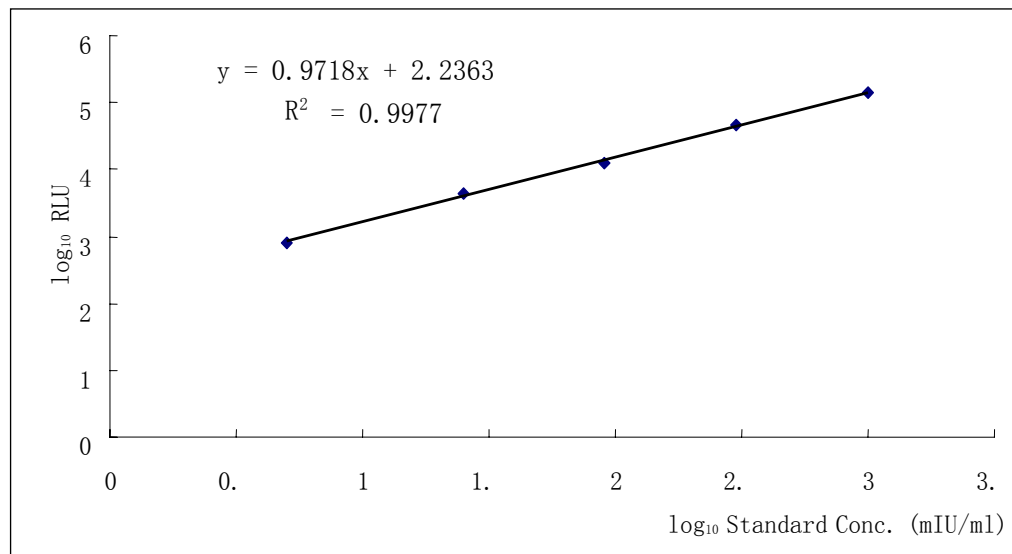
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On logarithmic graph paper plot the $\log_{10}$ RLU (ordinate) obtained from each reference standard against the common logarithm of corresponding concentration of Anti-HBs in mIU/ml (abscissa) and draw a calibration curve through the references standard points by connecting the plotted points with curved lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.
5. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF CALIBRATOR CURVE

A typical calibration curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

Anti-HBs (mIU/ml)	RLU
0	53.69
5	772.475
25	4502.145
90	12394.5
300	46842.7
1000	136804.9

$y = 0.9718x + 2.2363$

$R^2 = 0.9977$

EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. The concentration of Anti-HBs in the sample is determined using a previously generated calibration. If the

concentration of the sample is greater than or equal to 10mIU/ml, the sample is considered reactive for Anti-HBs.

PERFORMANCE
A. Sensitivity

In studies performed at AUTOBIO laboratories, using an Anti-HBs reference panel, sensitivity results calculated by linear regression was 2mIU/mL. A total of 406 specimens from 256 HBV vaccine recipients, 45 individuals recovered from HBV infection, and 105 individuals at risk for HBV infection were tested. Of the 406 specimens, 367 (90.39%) were repeatedly reactive and positive by supplemental testing.

B. HBV Vaccine Recipient Serial Bleed Panels

A total of 100 specimens comprising 15 serial bleed panels from HBV vaccine recipients were tested. The vaccine was administered in three injections over a six-month period. All specimens drawn one month following the third and final injection were reactive by the AUTOBIO Anti-HBs assay.

C. Correlation

AUTOBIO Anti-HBs was compared to a commercially available assay for correlation using the Passing-Bablok Regression method and Spearman Rank correlation. The comparison was made using results from 180 specimens from HBV vaccine recipients and individuals who have recovered from HBV infection. The correlation coefficient was 0.922, the slope was 1.03 and the intercept was -32.7.

D. Specificity

Three clinical sites tested a total of 8915 serum and plasma specimens from the following categories: volunteer whole blood donors, matched serum and plasma pairs, random hospital patients, medical conditions unrelated to HBV infection and potentially interfering substances. A total of 4490 (50.36%) of the 8915 specimens were repeatedly reactive, and 4423 (98.51%) of the 4490 specimens were positive by supplemental testing.

NOTE: Medical conditions unrelated to HBV infection and potentially interfering substances, included the following: anti-CMV (10), anti-EBV (10), anti-HSV (10), anti-HAV (20), anti-HCV (10), anti-HIV-1 (10), HBV vaccine recipients (30), rubella antibody (10), toxoplasma antibody (10), E. coli infections (10), yeast infections (10), syphilis (30), anti-nuclear antibody (10), rheumatoid factor (10), multiple myeloma (10), multiparous females (10), pregnant females (80), and alcoholic liver disease (10).

E. Precision
a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low Titer (41.44mIU/ml)	20	26.77	1.83	6.83
Medium titer (26.25mIU/ml)	20	143.02	6.88	4.81

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low Titer (41.44mIU/ml)	10	26.93	2.64	9.80
Medium titer (26.25mIU/ml)	10	146.22	8.16	5.59

F. High Dose Hook Effect

No hook effect occurred with Anti-HBs concentration up to 10000mIU/ml. However, since high responders may show extremely high levels, false low results due to a high dose hook effect may be seen in specimens from these patients. In order to avoid reporting misleading low results due to a hook effect at higher concentrations, particularly in patients for whom markers are being measured for the first time, or when very high Anti-HBs values may be expected, it is recommended to assay specimens at dilutions (diluted 1:100 with physiological saline).

G. Overall Specificity and Sensitivity

Overall specificity and sensitivity were estimated from the results of 9321 serum and plasma specimens, which were tested with AUTOBIO Anti-HBs at three clinical sites. The overall specificity was estimated to be 98.12% (4390/4474). The overall sensitivity was estimated to be 99.07% (4802/4847)
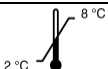
LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Human serum (including serum collected in serum separator tubes) or plasma collected in potassium EDTA, sodium citrate, and sodium heparin may be used in the AUTOBIO Anti-HBs assay. Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient samples.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
⧖	**USE BY**
🏭	**MANUFACTURER**
∑	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
🌡 8 °C / 2 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
📖	**CONSULT INSTRUCTIONS FOR USE**

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