IVD

HEPATITIS B SURFACE ANTIGEN (HBSAG) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0310-2

INTENDED USE

The AUTOBIO hepatitis B surface antigen (HBsAg) chemiluminescence immunoassay (CLIA) is intended for the quantitative determination of HBsAg concentration in human serum and plasma.

INTRODUCTION

Hepatitits B is a disease caused by viral infection. The route of infection can be improper needle puncture, blood transfusion or even taking contaminated food or water. Hepatitis B is an immune disease. Invasion of the human body by Hepatitis B virus induces autoimmune reactions, which damage the liver [1-2].

Three types of virus-derived particles can be identified in HBV-infected individuals. First, HBV virions or Dane particles, comprising an outer envelope composed of a mixture of glycoproteins, known collectively as HBsAg.This envelope surrounds an inner nucleocapsid made up of the hepatitis B core antigen (HBcAg), which contains the circular partially double-stranded DNA genome. Secondly, spherical particles of nearly 22 nm in diameter and, thirdly, filamentous structures of nearly 20 to 22 nm in diameter and of variable length can also be identified. The spherical and filamentous particles consist of host cell lipid in combination with the virus-derived envelope HBsAg .The particles are found in a 10^4 to 10^6 fold excess over the HBV virions, which is why HBsAg has been proven to be such a good marker for hepatitis B infection. The purified 22-nm particles are noninfectious but highly immunogenic, and are the active component of the original hepatitis B vaccine. There are six immunologic markers of HBV: HBsAg, HBcAg, HBeAg and their respective antibodies. The HBsAg however is the first marker to appear in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier. The development of serological assays to detect hepatitis B surface antigen (HBsAg) has played a major role in the diagnosis of hepatitis B virus (HBV) infection. With other hepatitis B serological assays, a diagnosis of acute or chronic HBV infection, past infection, or successful vaccination can be determined.[1-2]

Recent studies[3-5] showed that there were many clinical applications of quantitative determination of HBsAg concentration in human serum or plasma. Firstly,the quantification of HBsAg provided a means of monitoring the effectiveness of antiviral therapy and detecting the early development of antiviral drug resistance .Secondly,the HBsAg concentration was significantly higher in HBeAg-positive than in HBeAg-negative patients, and there was a significant correlation between the HBsAg concentration and HBV DNA level. After the start of lamivudine therapy, the HBV DNA levels fell rapidly in all patients and so did the serum HBsAg concentrations. Also, in some patients, the increase in HBsAg preceded the increase in HBV DNA.Thirdly,the quantitative determination of HBsAg and anti-HBc/IgM provided additional information, and may be useful in the differential diagnosis of acute and chronic HBV infections and in the follow-up of chronically infected patients. Fourthly, at baseline, the serum HBsAg levels correlated well with the cccDNA. In conclusion, HBsAg quantitation can be a surrogate marker for viral load during the management of chronic HBV infection.

PRINCIPLE OF THE TEST

The HBsAg CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one anti-HBs monoclonal antibody (anti-HBS Mab) for solid phase (microtiter wells) immobilization and another anti-HBs polyclonal antibody (anti-HBS Pab) as antibody-enzyme (horseradish peroxidase) conjugate reagent. The HBsAg present in the reference standards and serum or plasma are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-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 HBsAg present in the specimen.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to Hepatitis B Surface Antigen (anti-HBs MAb) (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-HBs MAn in Stabilizing Buffer (1 vial, 6.0ml)
3. Reference Standards: 0 （liquid,1vial,0.5ml）, 0.1(lyophilized), 0.5(lyophilized), 3(lyophilized), 20(lyophilized) and 180(lyophilized)ng/ml HBsAg 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 calibrators 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 HBsAg 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 diluted of human plasma known to be positive for Hepatitis B surface antigen, 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 Standards with 0.5 ml distilled water and the Reference Standards of 0 ng/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 Washing Buffer: add 1 bag of PBS-T Powder to 500 ml of distilled water, and mix well. The washing 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 HBsAg 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 washing buffer either manually or with an automatic washer.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense25μl of Substrate A, then 25μl 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.

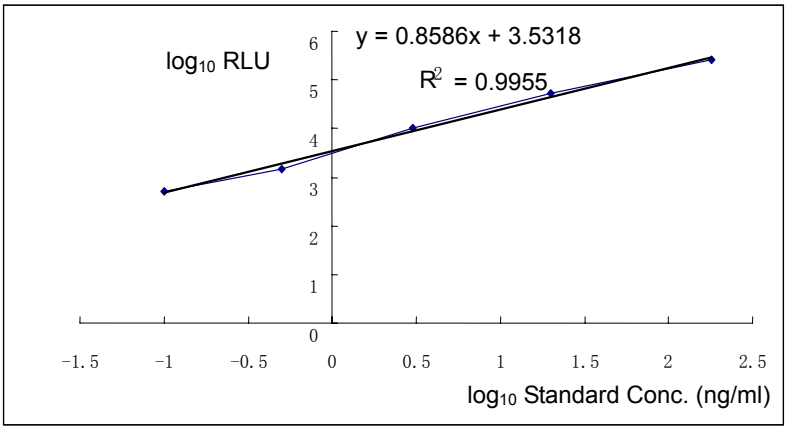
CALCULATION OF RESULTS

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 HBsAg in ng/ml (abscissa) and draw a calibration curve through the reference 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.

HBsAg (ng/ml)	RLU
0	27.04
0.1	497.06
0.5	1496.09
3	10070.4
20	52326.7
180	258181.7



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. The concentration of HBsAg in the sample is determined using a previously generated calibration. If the concentration of the sample is greater than or equal to 0.15ng/ml, the sample is considered reactive for HBsAg.

PERFORMANCE

A. Sensitivity

The AUTOBIO HBsAg assay has a sensitivity less than 0.15ng/ml.

NOTE: In studies performed at AUTOBIO laboratories, using a HBsAg ad/ay reference panel, sensitivity results calculated by linear regression have ranged from 0.2ng/ml to 0.5ng/ml. A total of 602 serum and plasma specimens from 401 individuals known to be positive for HBsAg, 22 individuals with acute HBV infection, 75 individuals with chronic HBV infection, and 304 individuals at increased risk for HBV infection were tested. Of the 602 specimens, 512 (85.05%) were repeatedly reactive and confirmed positive by specific antibody neutralization.

B. Specificity

A total of 8915 serum and plasma specimens from voluntary whole blood donors, a low prevalence population for HBV infection, were evaluated at three clinical sites. The initial and repeat reactive rates were 5.63% (502/8915) and 5.61% (500/8915), respectively. Of the 500 repeatedly reactive specimens, the presence of HBsAg was confirmed by specific neutralization with anti-HBs in one specimen. Thirty eight of 830 specimens obtained from hospital patients were repeatedly reactive and confirmed positive for HBsAg. In 42 matched serum and plasma pairs, none of the specimens were repeatedly reactive. Only the matched plasma specimens are included in the AUTOBIO HBsAg specificity calculation. In 300 specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances, six specimens were repeatedly reactive, and five of the six specimens were confirmed positive for HBsAg.

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).

C. 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（0.24ng/ml）	20	0.26	0.024	9.23
Medium titer（1.91ng/ml）	20	1.94	0.112	5.77

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 (0.24ng/ml)	10	0.22	0.028	13.00
Medium titer(1.91ng/ml)	10	1.21	0.120	9.89

D. High Dose Hook Effect

No hook effect occurred with HBsAg concentration up to 50000 ng/ml. However, since acute HBV infection patients 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 HBsAg values may be expected, it is recommended to assay specimens at two dilutions (*i.e.* neat and diluted 1:1000 with physiological saline).

***E.* Overall Specificity and Sensitivity**

Overall specificity and sensitivity were estimated from the results of 10000 serum and plasma specimens, tested with Autobio HBsAg at three clinical sites. The overall specificity was estimated to be 99.89% (9045/9055). The overall sensitivity was estimated to be 99.58% (941/945)

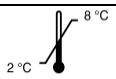
LIMITATIONS

6. 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.
7. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. Additional clinical or diagnostic information may be required to determine patient status.
8. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
9. 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

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

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Antigen, Immunoglobulin G, By Means of Enzyme-Labelled Antigen and Antibody-Coated Tubes. Biochem Biophys Acta 1971;251:427-34.

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