IVD

ANTIBODY TO HEPATITIS B CORE ANTIGEN (ANTI-HBC)

CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0314-2

INTENDED USE

The AUTOBIO antibody to hepatitis B core antigen (anti-HBc) chemiluminescence immunoassay (CLIA) is intended for the quantitative determination of anti-HBc 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 by 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.

The determination of anti-HBc levels can be sued to examine the progress of Hepatitis B virus (HBV) infection. In acute case of Hepatitis B infection, anti-HBc is detectable in the blood shortly after the apperance of HBsAg. HBsAg levels often decline before the appearance of anti-HBs. During this interval between the decline of HBsAg and the rise in anti-HBs, total anti-HBc may be the only reliable marker of HBV infection.

In chronic HBV infections, HBsAg rises during the incubation phase and may persist for years. Anti-HBc also appears during this early phase and reaches high titers which may persist for years. In asymptomatic HBV infections, HBsAg and HBeAg are present only briefly and are quickly followed by the appearance of anti-HBs and anti-HBc. Therefore in such patients. Sometimes the only evidence of an infection may be the detection of Anti-HBs and Anti-HBc.

PRINCIPLE OF THE TEST

Anti-HBc test is a competitive enzyme immunoassay in which anti-HBc from specimens and reference standards compete with a constant amount of horseradish peroxidase (HRP) conjugated anti-HBc for a limited number of HBcAg coated onto the wells. Anti-HBc reference standards and serum or plasma specimens are added to the microtiter wells together with HRP conjugated anti-HBc. After incubation, anti-HBc in specimen, if present, compete with constant amount of HRP-conjugated anti-HBc for limited amount of HBcAg added into the wells. The unbound antibody-enzyme labels are removed by washing. The chemiluminescence reaction is developed when the CLIA substrate is mixed with HRP-conjugated-anti-HBc. The Related Light Unit (RLU) is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Anti-HBc in the sample. By reference to a series of anti-HBc reference standards assayed in the same way, the concentration of anti-HBc in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate coated with hepatitis B core antigen (HBcAg) (1 plate, 96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled antibody to Hepatitis B core antigen (anti-HBc) in Stabilizing Buffer (1 vial, 6.0ml)
3. Reference Standards: 0, 2, 5, 12, 30 and 60NCU/ml anti-HBc in Stabilizing Buffer (6 vials, 0.5ml/ea)
4. Substrate A: (1 vial, 3.5ml)
5. Substrate B: (1 vial, 3.5ml)
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 20-200µl, 100-1000µl (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. 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 two 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 HBeAg 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.
6. Do not use heat-inactivated specimens.

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 Hepatitis B c antibody, 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 Calibrators 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. To prepare wash buffer: add 1 bag of PBS-T Powder into 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 washing 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 Substrate 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.

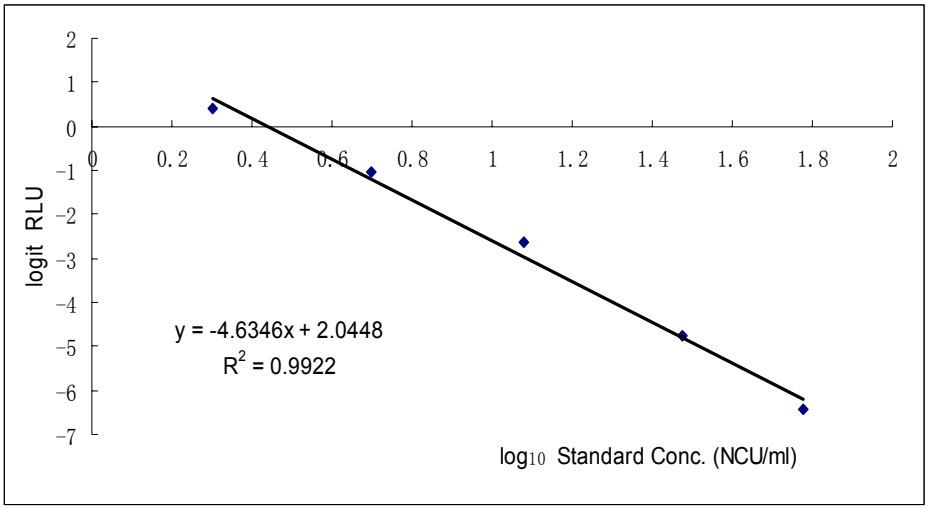
CALCULATION OF RESULTS

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 logit RLU (ordinate) obtained from each Reference Standard against the common logarithm of corresponding concentration of anti-HBc in NCU/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 STANDARD 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-HBc (NCU/ml)	RLU
0	310611.9
2	185115.15
5	80827
12	20542.7
30	2597.675
60	505.185



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance.
The concentration of anti-HBc in the sample is determined using a previously generated calibration. If the concentration of the sample is greater than or equal to 3NCU/ml, the sample is considered reactive for anti-HBc.

PERFORMANCE

A. Sensitivity

The AUTOBIO anti-HBc assay has a sensitivity lower than 3.0NCU/ml. A study was performed in which a total of 450 specimens, which were pre-characterized reactive for Anti-HBc, were all reactive by AUTOBIO

anti-HBc. A study was performed in which a total of 93 specimens from individuals clinically or serologically classified with different stages of HBV infection were tested by AUTOBIO anti-HBc. All of these specimens were reactive.

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 51.00% (4590/8915) and 51.99% (4569/8915), respectively. Of the4569 repeatedly reactive specimens, 228 were positive for HBsAg , 4291 were positive for Anti-HBs ,and 4489 of the 4569 specimens were positive by supplemental testing.556 of 830 specimens obtained from hospital patients were repeatedly reactive and 546 specimens was positive by supplemental testing. In 42 matched serum and plasma pairs, 15 specimens were repeatedly reactive. Only the matched plasma specimens are included in the AUTOBIO Anti-HBc specificity calculation. In 300 specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances, 97specimens were repeatedly reactive, and 96 of 97specimens was 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).

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(1.99NCU/ml)	20	1.69	0.15	8.88
Medium titer (5.5NCU/ml)	20	5.97	0.43	7.20

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(1.99NCU/ml)	10	2.05	0.26	12.68
Medium titer (5.5NCU/ml)	10	5.90	0.499	8.46

***D.* Accuracy**

Overall specificity and sensitivity were estimated from the results of 10087 serum and plasma specimens, tested with Autobio Anti-HBc at three clinical sites. The overall specificity was estimated to be 95.20% (4519/4747). The overall sensitivity was estimated to be 98.07% (5237/5340)

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. 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.15,16 Additional clinical or diagnostic information may be required to determine patient status.
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
Σ	**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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