



HEPATITIS B E ANTIGEN (HBEAG) CHEMILUMINESCENCE

IMMUNOASSAY KIT

Catalog No. CL0312-2

INTENDED USE

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

INTRODUCTION

Hepatitis 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. ¹⁻²

The Hepatitis e antigen (HBeAg) is a peptide and normally detectable in the bloodstream when the hepatitis B virus is actively reproducing, this in turn leads to the person being much more infectious and at a greater risk of progression to liver disease. The exact function of this non structural protein is unknown, however it is thought that HBeAg may be influential in suppressing the immune systems response to HBV infection. HBeAg is generally detectable at the same time as HBsAg and disappears before HBsAg disappears. The presence of HBeAg in chronic infection is generally taken to indicate that HBV is actively reproducing and there is a higher probability of liver damage. In acute infection HBeAg is generally only transiently present. However mutant strains of HBV exist that replicate without producing HBeAg. ¹⁻²

The present studies⁵⁻⁸ suggest that quantitation of HBeAg in serum may be a useful adjunct to serum HBV DNA assays to fully evaluate the response to antiviral therapy in patients treated with peginterferon. Among patients who ultimately achieved HBeAg seroconversion, levels of HBeAg decreased consistently and remained at the lowest levels during the follow-up period. In contrast, a rebound was observed in patients who failed to achieve seroconversion after treatment was discontinued. In addition, analysis of HBeAg levels after 24 weeks of treatment provided a better indicator of nonresponse to peginterferon alfa-2a than did a similar analysis of HBV DNA levels. Quantifying HBeAg during therapy also allowed demarcation of late responders from nonresponders, a subgroup of patients who were not differentiated by changes in HBV DNA levels. Among late HBeAg responders, HBV DNA levels were lower than for non-HBeAg responders. The analysis we conducted in this study suggests that quantification of HBeAg may be a useful clinical tool for predicting the absence of response to peginterferon alfa-2a in an individual patient. Our results suggest that a critical level of reduction of HBeAg may be definable which, if not achieved by patients undergoing treatment, may indicate that a discontinuation of peginterferon monotherapy should be considered.

PRINCIPLE OF THE TEST

The HBeAg CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal antibody to hepatitis B e antigen (anti-HBe MAb) for solid phase (microtiter wells) immobilization and another anti-HBe MAb as antibody-enzyme (horseradish peroxidase) conjugate reagent. The HBeAg 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 HBeAg present in the specimen.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to Hepatitis B e Antigen (anti-HBe MAb) (1 plate, 96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-HBe MAb in Stabilizing Buffer (1 vial, 6.0ml)
3. Reference Standards: 0, 0.25, 1, 5, 25 and 100NCU/ml HBeAg in Stabilizing Buffer (6 vials, 0.5ml/ea)

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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 20-200 μ l, 100-1000 μ l (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 $^{\circ}$ C 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 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 HBeAg assay. Liquid anticoagulants may have a dilution effect resulting in false 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 $^{\circ}$ C. For longer storage, freeze the specimens at -20 $^{\circ}$ C. 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 e 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 $^{\circ}$ C) prior to use.
2. To prepare wash buffer: add 1 bag of the 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 HBeAg 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°C 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 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.

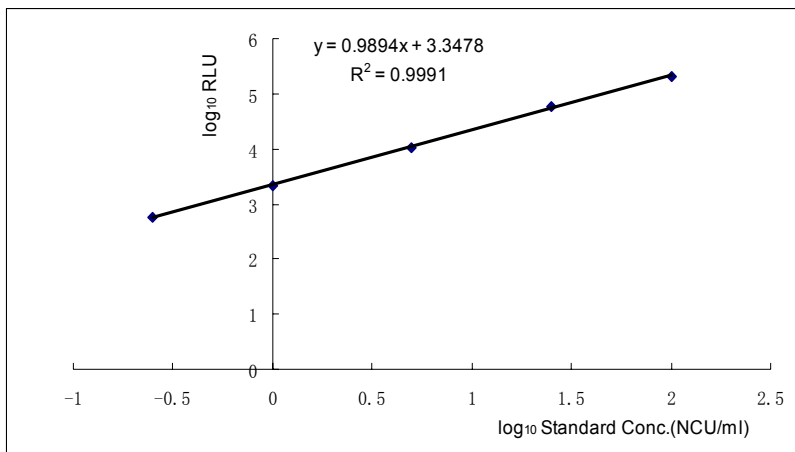
CALCULATION OF RESULTS

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 log₁₀ RLU (ordinate) obtained from each Reference Standard against the common logarithm of corresponding concentration of HBeAg 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.

HBeAg (NCU/ml)	RLU
0	107.945
0.25	588.31
1	2106.135
5	10494.745
25	59716.25
100	202668.9



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. The concentration of HBeAg in the sample is determined using a previously generated calibration. If the concentration of the sample is greater than or equal to 0.25NCU/ml, the sample is considered reactive for HBeAg.

PERFORMANCE

A. Sensitivity

The AUTOBIO HBeAg assay has a sensitivity lower than 0.25NCU/ml. A study was performed in which a total of 235 specimens, which were pre-characterized reactive for HBeAg and HBsAg, were all reactive by AUTOBIO HBeAg. 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 HBeAg. Twenty-nine out of 39 acute specimens were reactive and 10 were nonreactive. Out of 61 chronic specimens, 20 were reactive and 41 were nonreactive.

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 0.998% (89/8915) and 0.976% (87/8915), respectively. Of the 87 repeatedly reactive specimens, were also positive for HBsAg and 86 of the 87 specimens were positive by supplemental testing. Twenty of 830 specimens obtained from hospital patients were repeatedly reactive and was positive by supplemental testing. In 42 matched serum and plasma pairs, none of the specimens were repeatedly reactive. Only the matched plasma specimens are included in the AUTOBIO HBeAg specificity calculation. In 300 specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances, two specimens were repeatedly reactive, and one of the two specimens 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(0.76NCU/ml)	20	0.79	0.071	8.86
Medium titer (4.52NCU/ml)	20	4.68	0.30	6.41

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(0.76NCU/ml)	10	0.80	0.085	10.06
Medium titer (4.52NCU/ml)	10	4.68	0.36	7.69

D. High Dose Hook Effect

No hook effect occurred with HBeAg concentration up to 10000NCU/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 HBeAg values may be expected, it is recommended to assay specimens at two dilutions (i.e. neat and diluted 1:100 with physiological saline).

E. Accuracy

Overall specificity and sensitivity were estimated from the results of 10000 serum and plasma specimens, tested with Autobio HBeAg at three clinical sites. The overall specificity was estimated to be 99.99% (9050/9055). The overall sensitivity was estimated to be 99.79% (943/945)

LIMITATIONS






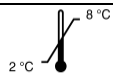


1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a

- 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. 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

	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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