IVD

Hepatitis B e Antigen (HBeAg) ELISA

Catalog No: E0317

96 tests

INTENDED USE

The Autobio HBeAg ELISA is designed for the qualitative determination of hepatitis B e antigen (HBeAg) concentration in human serum or plasma specimens.

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 has become a significant problem for public health management. Almost one in every ten adults, who have been infected by Hepatitis B Virus (HBV), develops some form of chronic liver disease and becomes a long-term carrier of HBV. Screening for Hepatitis B is therefore urgently needed.

Hepatitis B is an immune disease. Invasion of the human body by HBV induces auto-immune reactions which damage the liver. The components of the virus (antigens) and the host responses (antibodies), the so called immunologic markers have often been used as diagnostic tools.

There are six immunologic markers of HBV: HBeAg, HBcAg, HBsAg and their respective antibodies. HBeAg however is detected only in HBsAg positive sera. Its presence coincides with the rapid propagation of HBV and high infectivity. It is also a marker of questionable prognosis including the development of chronic Hepatitis. On the contrary, anti-HBe represents minimum viral replication and greatly reduced infectivity. When a patient changes from HBeAg to its antibody, he or she is likely to enter convalescent stage. But it is also possible that patients with anti-HBe are long-term carriers of HBV. Nevertheless, patients with anti-HBe generally have optimistic prognosis.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

The AUTOBIO HBeAg ELISA is based on the sandwich method. The wells of the micro titer plate are coated with monoclonal antibodies specific to HBeAg (anti-HBe MAb) which forms stable complexes with HBeAg present in the serum or plasma samples pipetted into the wells. The enzymatic tracer, horseradish peroxidase (HRP) labeled anti-HBe IgG is added to the wells and where the antigen-antibody complex is present, the enzyme conjugate binds to the antigen eventually forming the following complex: antibody-antigen-antibody-HRP. The activity of the HRP is then revealed by the addition of the chromogen and substrate solutions which generates a blue color which then turns to yellow after terminating the reaction with acid.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with anti-HBe MAb coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: HRP labeled anti-HBe in Stabilizing Buffer (1 vial, 7.5ml).
3. Negative Control: Heat inactivated healthy human sera diluted in buffer (1 vial, 0.8ml).
4. Positive Control: human sera containing purified and inactivated HBeAg and diluted by normal human serum. (1 vial, 0.8ml).
5. Wash Fluid Concentrate: PBS-Tween (1 bottle, 30ml, 20X)
6. Substrate Solution: Hydrogen peroxide (1 vial, 7.5ml)
7. Chromogen Solution: Tetramethylbenzidine (TMB) (1 vial, 7.5ml)
8. Stop Solution: 1.0M H_2SO_4 (1 vial, 7.5ml)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water

3. Vortex mixer
4. Absorbent paper or paper towel
5. Incubator
6. Disposable reagent troughs
7. Instrumentation
    1. Automated microplate strip washer
    2. Microplate reader

    or

    3. Fully automated microplate processor

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout 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. All opened components will remain stable for at least 2 months, or until the labeled expiration date, whichever is earlier, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Plasma samples may be used but serum is the recommended sample type for this assay.
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℃. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20℃ or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.

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. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
4. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
5. Please read the instruction for use carefully prior to use, and carry out the test strictly according to time and temperature indicated on the instructions.
6. Read the result within 20 minutes after terminating the test.
7. Do not use sodium azide as preservative in the samples.
8. Components with different lot numbers are not allowed to be exchanged.
9. The activity of enzyme conjugate reagent is easily destroyed by metal and other substances. Avoid exposing Chromogen Solution to direct sunlight, metal or oxidants.
10. Avoid microbial contamination of reagents.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18 – 25℃) prior to use,place at room temperature for at least 30 minates,place at room temperature for at least 30 minates.
2. Adjust the incubator to 37℃.
3. Add 50ml of Wash Solution Concentrate to 1000ml of distilled water, and mix well with a magnetic stirrer. The Wash Solution is stable at room temperature for two months.

IMPORTANT NOTES

1. Do not use reagents after expiration date.

2. Do not mix or use components from kits with different lot numbers.
3. Do no reuse the plate covers.
4. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
5. Replace caps on reagents immediately. Do not switch caps.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Leave 1 well for the blank, 5 additional wells for the controls and 1 well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve 1 well for blank, add 50 μL of Negative Control to each of the 3 following wells, 50 μL of Positive Control to each of the next 2 wells and 50 L of each sample to each of the remaining wells. Then add 50 μL of Enzyme Conjugate Reagent into each well except the blank well.
4. Thoroughly mix for 30 seconds and cover the top of the microtiter plate with plate covers. It is important to have complete mixing in this step.
5. Incubate the plate at 37°C for 30 minutes.
6. Tear the plate covers out of the plate. Wash the microtiter plate 6 times with an automated microplate strip washer, then strike the plate sharply onto absorbent paper to remove residual water droplets.
7. Dispense 50 μL of Chromogen Solution into each well.
8. Dispense 50 μL of Substrate Solution into each well. Gently mix horizontally for 15 seconds.
9. Incubate the plate at 37°C in the dark for 10 minutes without shaking.
10. Terminate the reaction by adding 50 μL of Stop Solution to each well. Mix gently.
11. Immediately after mixing, read the absorbance of each well at 450 nm with an microplate reader using 620 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

INTERPRETATION OF RESULTS

1. Test is valid if

Mean absorbance of negative control is lower than 0.10.

Mean absorbance of positive control is equal to or higher than 0.6.

2. Calculation of cut-off value

   Cut-off value = 2.1×NCx (Mean absorbance of Negative Control)
   If the absorbance of Negative Control is less than 0.05, 0.05 should be used for calculation

3. Interpretation of results

Any specimen with an absorbance equal to or greater than the cut-off value is considered positive.
Any Specimen with an absorbance less than the cut-off value is considered negative.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity conforms to the national requirements after assaying a series of national standard sensitivity references of P.R. China.

2. Specificity

No evident cross reactions were observed with HAV, HCV and HIV markers. The assay was not interfered by RF factors, specimens containing high bilirubin concentrations, grossly hemolytic and lipemic specimens.

3. Precision

After 10 replicate tests, the precision was calculated to be ≤15%.

4. Negative Concordance

The concordance with China's national negative reference is 100%.

5. Positive Concordance

The concordance with China's national positive reference is 9/10.

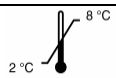
LIMITATIONS

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results, in particular, correct sample and reagent pipetting, along with careful washing and timing of incubation steps is essential for an accurate, reproducible detection of HBeAg.
2. Use fresh plasma samples, or samples frozen and thawed only once. Samples degradation as well as multiple freeze-thaw cycles may cause erroneous results. Do not use heat inactivated samples.

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**
	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

for orders and inquiries, please contact

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