Antibody to Hepatitis B e Antigen (anti-HBe) ELISA

Catalog No: E0318

96 tests

INTENDED USE

The Autobio anti-HBe ELISA is intended for the qualitative detection of antibodies to hepatitis B e antigen concentrations in human serum or plasma specimens.

INTRODUCTION

The presence of antibody against hepatitis B viral e antigen is used as an indicator for early HBs antigenemia before the peak of viral replication and early convalescence when HBeAg has declined below detectable levels. It is also useful to confirm a seroconversion. The seroconversion from HBeAg positivity to anti-HBe positivity indicates a reduced level of infectious virus because virus replication has decreased.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

Anti-HBe test is a competitive enzyme immunoassay in which monoclonal antibody (MAb) to hepatitis e antigen (HBeAg) is pre-coated on microtiter wells, then HBeAg is attached to the solid phase through the pre-coated anti-HBe. Anti-HBe in specimens competes with a constant amount of horseradish peroxidase (HRP) conjugated anti-HBe MAb for the limited number of HBeAg on the wells. The unbound materials will then be washed away and the chromogen substrate solution containing hydrogen peroxide is added to the wells for color development. Thus, the amount of HRP-conjugated anti-HBe bound to the well is inversely proportional to the concentration of anti-HBe in the specimen. The absorbance of controls and specimens is determined using a microplate reader with the wavelength set at 450 nm and 620nm.

MATERIALS PROVIDED

1. Coated Wells: microplate with anti-HBe MAb coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: HRP labeled anti-HBe MAb 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 anti-HBe 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.
3. Return other unused components to 2 – 8℃. Use within the expiry date.

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. Ensure the specimens are not decayed prior to testing.
5. Avoid grossly hemolytic, lipemic or turbid samples.
6. Store samples at 2 – 8℃. Samples to be transported or stored for more than 24 hours should be stored frozen at - 20℃ or colder. Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed and brought to room temperature before being assayed.
7. Do not add sodium azide into the specimen as a preservative.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. The assay must be conducted away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as 84 disinfectant, sodium hypochlorite, acid, alkaline, acetaldehyde and so on, or containing dust.
4. Micropipette tips are not interchangeable to eliminate cross contamination.
5. Specimens added must be mixed thoroughly. The presence of bubbles must be eliminated.
6. The microplate must be washed completely. Each well must be fully injected with Wash Fluid. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, all wells must be dried. The microplate should be stroked onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate stripwasher.
7. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. Controls contain human source materials, which have been tested negative for HIV and HCV. However, all specimens must be regarded as potentially infectious. Waste material must be disposed of safely according to relevant local and national requirements.
8. 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,placeat room temperature for at least 30 minates.
2. Dilute Wash Fluid Concentrate 20 folds in distilled water.
3. Adjust the incubator to 37℃.

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 pipetting is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting 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 RLU readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Write down the relative numbers of specimens and wells on the data sheet. 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 zip-lock bag (included in the kit) which contains desiccant.
3. Reserve 1 well for blank, add 50 l of the Negative Control to each of 3 wells, 50 l of the Positive Control to each of 2 wells and 50 l of sample to each of the other wells.Then add 50μl of Enzyme Conjugate Reagent into each well except the blank well.

   Notes: use an individual tip for each pipetting to avoid cross contaminations.
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. Rinse the microtiter wells 6 times with wash solution in the washer, then strike the plate sharply onto absorbent paper to remove residual water droplets. The microtiter plate could also be washed 5 times by hands (Remove the incubation mixture by flicking plate contents into a waste container and flick to dry. Filling each well with wash solutions (about 350μl per well). Discard the wash solution after 5~10 seconds of the final washing, strike the plate sharply onto absorbent paper to remove residual water droplets. Wash the wells 5 times with the wash solution by repeating this step).
7. Dispense 50μl of the Chromogen Solution into each well.
8. Dispense 50μl of the Substrate Solution into each well. Gently mix for 15 seconds.
9. Incubate the plate at 37°C in the dark for 10 minutes without shaking.
10. Stop the reaction by adding 50μl of the Stop Solution to each well. Mix gently.
11. Immediately after mixing, read the absorbance of each well at 450 nm with an ELISA 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 if valid if

   Mean absorbance of Negative Controls is greater than 0.8.

   Mean absorbance of Positive Controls is less than 0.1.

2. Calculation of cut-off value

   Cut-off value = (mean absorbance of Negative Controls + mean absorbance of Positive Controls)/2
3. Interpretation of results

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

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity met 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 ≤20%.

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. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by a physician is needed for a final diagnosis.

2. This assay can only test plasma or serum samples. The reliability to test other body fluids is not confirmed.

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

Symbol	Meaning
LOT	**BATCH CODE**
	USE BY
	MANUFACTURER
Σ	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
2 °C – 8 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

for orders and inquiries, please contact

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