IVD

Antibody to Hepatitis B Core Antigen (anti-HBc) ELISA

Catalog No: E0319

96 tests

INTENDED USE

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

INTRODUCTION

Hepatitits B (HB) is a viral infection, in which the route of infection can be sexual contact, improper needle puncture, blood transfusion or even contaminated food or water.

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 apperars 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 apperance 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.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

This assay is based on competitive ELISA. Recombinant hepatitis core antigen is pre-coated on microtiter wells. Anti-HBc in patient sample and Anti-HBc-Peroxidase conjugate (Anti-HBc-HRP, Enzyme tracer) compete for the limited binding sites of the recombinant Hepatitits B core antigen (ANcAg), coated onto the wells. After incubation, the unbound reagents are removed by washing and tetramethylbenzidine (TMB) solution containing hydrogen peroxide is added to the well. During the incubation, a blue color develops in proportion to the amount of Anti-HBc-HRP bound to the well. If a patient sample does not contain Anti-HBc, the quantity of Anti-HBc-HRP conjugate remaining bound to the solid phase immunocomplex is maximum and, therefore, the colour developed during the enzymatic reaction is quite intense. On the contrary, if the sample contains Anti-HBc, the mount of the Anti-HBc-HRP remaining bound is reduced and the color is weaker. Sample positivity or negativity is given by reference to a cut-off control serum.

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, place at 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 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. 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.
3. Thoroughly mix for 30 seconds and cover the microplate with a lid. It is important to have complete mixing in this step.
4. Incubate the plate at 37°C for 30 minutes.
5. Wash the microplate 6 times with an automated microplate stripwasher. After washing is completed, invert the microplate and tap out any residual Wash Fluid onto absorbent paper.
6. Dispense 50 μL of Chromogen Solution into each well.
7. Dispense 50 μL of Substrate Solution into each well. Gently mix horizontally for 15 seconds.
8. Incubate the plate at 37°C in the dark for 10 minutes without shaking.
9. Add 50 μL of Stop Solution to each well. Mix gently.
10. Immediately after mixing, read the absorbance of each well at 450 nm in a microplate reader using 620 – 630 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 14/15.

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 standard 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**

for orders and inquiries, please contact

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