IVD

Antibody to Hepatitis B Surface Antigen (anti-HBs) ELISA

Catalog No: E0316

96 tests

INTENDED USE

The Autobio anti-HBs ELISA is designed for the qualitative determination of antibodies to hepatitis B surface antigen (anti-HBs) concentration in human serum or plasma specimens.

INTRODUCTION

Anti-HBs titer can be determined to monitor the prognosis of patients recovering from the hepatitis B viral infection. It also can be used as an indicator of prior exposure to Hepatitis B viruses.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

The anti-HBs ELISA is a solid-phase simultaneous immunoassay to detect antibodies against HBsAg. Microwells are coated with hepatitis B surface antigen (HBsAg). A serum specimen is added to the microwells together with horseradish peroxidase (HRP) Labeled HBsAg. After incubation, the complex of antigen antibody-antigen (HRP-conjugated HBsAg, anti-HBs antibody and HBsAg on the wells) will be formed. Thus, the amount of HRP-HBsAg conjugate bound to the well is proportional to the concentration of anti-HBs in the specimen. The unbound enzyme conjugates will be washed away and then the chromogen and substrate solutions containing hydrogen peroxide is added to the wells. A blue color is developed in proportion to the amount of anti-HBs antibodies the specimens. The enzyme-substrate reaction is terminated with the addition of acid. The absorbance of controls and specimens is read in a microplate reader at the wavelength of 450 nm and 620nm.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate with naturally inactivated HBsAg coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: HRP labeled HBsAg 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-HBs 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 volumes (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..
2. Adjust the incubator to 37℃.
3. Carry out a 1:20 dilution of Wash Fluid Concentrate.

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 Posi-

tive Control to each of the next 2 wells and 50 μL 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 (including the blank 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
Example:
NCx = 0.032
Cut-off = 2.1 × 0.05= 0.105

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 reaches 10 mIU/mL.

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%.

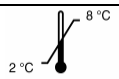
LIMITATIONS

1. Anti-HBs ELISA is limited to the detection of antibodies against HBsAg in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells before adding Wash Fluid.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test. A complete evaluation by a physician is needed for a final diagnosis.

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**

for orders and inquiries, please contact

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