IVD

Hepatitis B Surface Antigen (HBsAg) ELISA

Catalog No: E0315

96 tests

INTENDED USE

The Autobio HBsAg assay is designed for the qualitative determination of hepatitis B surface antigen (HBsAg) concentration in human serum or human plasma.

INTRODUCTION

Hepatitits B is a disease caused by viral infection. There are many routes of infection such as improper needle puncture, blood transfusion or even by taking contaminated food or water.

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. Hepatitis B has become a significant problem for public health management. Screening for hepatitis B is therefore urgently needed.

The components of the virus (antigens), the host responses (antibodies) and the so-called immunologic markers have often been used as diagnostic tools.

There are six immunologic markers of HBV: HBsAg, HBcAg, HBeAg and their respective antibodies. The HBsAg however is the first marker appearing in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier.

PRINCIPLE OF THE TEST

The Autobio HBsAg assay is a direct immunoenzymatic method of the "sandwich" type in which rarefied anti-HBs monoclonal antibody coated on microplate wells act as the capture antibody and anti-HBs antibodies marked with peroxidase serve as conjugate antibodies. If the sample added to the plate contains HBsAg, the antigen will bind to the antibody on the plate and forms the antigen-antibody complex. Then washing eliminates any unbound material, and anti-HBs conjugated with peroxidase is added to the well and allowed to react with the antigen-antibody complex. After a second incubation and subsequent washing, an enzyme substrate containing a chromogen is added. The substrate will develop a blue colour if the sample is positive for HBsAg. The blue colour changes to yellow after the stop solution is added. he intensity of the colour is proportional to the amount of HBsAg in the samples.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with rarefied monoclonal antibodies to hepatitis B surface antigen (anti-HBS Mab) coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labelled anti-HBs conjugate 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 HBsAg and diluted by normal human serum. (1 vial, 0.8ml).
5. Wash Solution 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 and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. 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 for this assay 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℃) before use, place at room temperature for at least 30 minates.
2. Adjust the incubator to 37℃.
3. Prepare Wash Solution: 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. 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 ziplock bag (included in the kit) which contains desiccant.
3. Reserve 1 well for blank, add 50μl of the Negative Control to each of 3 wells, 50μl 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 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

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%.

5. Positive Concordance

The concordance with China's national positive reference is 3/3.

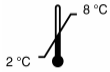
LIMITATIONS

1. As with other sensitive immunoassays, there is a possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the wash solution.
2. 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.
3. The test is for research use, further manufacturing and export only.

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 order and inquiries, please contact

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