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Hepatitis B Surface Antigen (HBsAg) ELISA

This kit is a qualitative detection of Hepatitis B surface antigen in human serum/plasma.

REF: BS.1/HBS01.096.0096 96 test

CLINICAL SIGNIFICANCE

Hepatitis B is caused by the Hepatitis B virus, an enveloped double-stranded DNA virus of the Hepadnaviridae family, and is a major cause of blood-transmitted hepatitis along with Hepatitis C. HBV infection can range from mild or inapparent disease to fulminant hepatitis and severe chronic liver diseases that may progress to cirrhosis and liver carcinoma. Diagnosis of HBV infection depends on identifying several serological markers during the incubation, acute, and convalescent phases. Among these markers, Hepatitis B surface antigen (HBsAg), formerly known as Australia antigen, is the most important envelope protein of HBV. HBsAg contains the common determinant “a” and is classified into two subgroups (ay and ad), with HBV having 10 major serotypes and four recognized HBsAg subtypes: adw, adr, ayw, and ayr. HBsAg appears 2–4 weeks before ALT levels become abnormal and 3–5 weeks before symptoms develop, making its serological detection by ELISA an important method for screening, diagnosis, and management of HBV infection.

METHOD PRINCIPLE

This kit uses double antibody sandwich ELISA principle. Purified hepatitis B surface antibody (HBsAb) is pre-coated on the microplate, the HBsAg in sample will combine with HBsAb first, then combine with enzyme-labeled HBsAb complex, and shows blue color in the microplate. This kit is used for the specific detection of hepatitis B surface antigen (HBsAg) in human serum/plasma.

COMPONENTS

Materials provided with the kit: (Components 96T/Box)

Components	96 T/Box	
Coated Plate	1 bag	96 wells
Enzyme Conjugate	1 vial	6.0 mL
Wash Buffer (40X)	1 vial	50 mL
Substrate A	1 vial	12 mL
Stop Solution	1 vial	6.0 ml
HBsAg calibrator 0 IU/ml	1 vial	1.0 ml
HBsAg calibrator 0.5 IU/ml	1 vial	1.0 ml
HBsAg calibrator 2 IU/ml	1 vial	1.0 ml
HBsAg calibrator 10 IU/ml	1 vial	1.0 ml
HBsAg calibrator 50 IU/ml	1 vial	1.0 ml
HBsAg calibrator 100 IU/ml	1 vial	1.0 ml

Note: different batches of reagent kit, and different components cannot be exchanged for use.

PRECAUTIONS AND WARNINGS

Good Laboratories practices using appropriate precautions should be followed in:

Wearing personnel protective equipment (overall, gloves, glasses,..).

In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.

2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot.

Never use reagents beyond their expiry date stated on labels or boxes.

3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.

4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.

5. Please put the unused plate back into the bag, and store at 2-8°C.

6. Operate strictly according to the instruction, control of reaction time and temperature strictly.

7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.

8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.

STORAGE AND EXPIRY

Store at 2-8°C. DO NOT FREEZE. Valid for 12 months. Once open, stable for 1 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

SPECIMEN COLLECTION AND PRESERVATION

1. No special patient’s preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the specimens are clear and not contaminated by microorganisms.

2. Highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. HBsAg ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

Procedure

1. All reagents should be allowed to reach room temperature for 15 minutes before use.
2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.
3. The sample should be corresponding to the number of microplate, each plate should be provided with negative control 3 wells, positive control 2 wells and blank control 1 well. (If detect with dual wavelength detection, setting no blank control well is allowed).
Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross contamination.
4. Add 20 µL Sample Diluent in the corresponding well (Do not add in the blank well, negative control wells and positive control wells).
5. Add 100 µL sample in the corresponding well, mix thoroughly by using the pipette, add 100 µL negative control and positive control to negative control wells and positive control wells (Do not add in the blank well) respectively.
6. Shake for 30 seconds with an oscillator (This step is very important). Incubate at 37°C for 60 minutes with the sealing plate membrane sealing the plate.
7. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.
8. Respectively adding Enzyme Conjugate 50 µL (Do not add in the blank well). Mix gently by shaking. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.
9. Repeat the wash step for 5 times as in step 7.
10. Add Substrate A (50 µL) and Substrate B (50 µL) (Do not add in the blank well). Mix gently by shaking. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.
11. Add 50 µL Stop Solution to each well (Do not add in the blank well). Mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm.If a dual filter instrument is used, set the reference wavelength at 630 nm. Set no blank wells is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction
with the patient clinical information.
2. The reagent is a qualitative reagent, and cannot be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PERFORMANCE CHARACTERISTICS

Negative Specificity: The coincidence rate should be 20/20 when detecting national negative quality control samples with ELISA kits of HBsAg.
Positive Specificity: The coincidence rate should be 3/3 when detecting national positive quality control samples with ELISA kits of HBsAg.
Limits Detection: Using National or Enterprise's sensitivity control for inspection, the cut-off value should be: adr subtype: ≤0.1 IU/mL, adw subtype: ≤0.1 IU/mL, ay subtype: ≤0.2 IU/mL.
Precision: Intra-assay: CV%≤15%; Precision between batches: CV%≤20%.

INTERPRETATION OF RESULTS

Colorimetry: Read the sample's optical density (OD) at 450nm with a microplate reader.
Mean negative control OD value ≤ 0.1 and positive control OD value ≥0.8, the test is valid, otherwise the test is invalid.
Cut-Off value (C.O.) = Mean negative control OD value x 2.1 (Calculated by 0.05 when Mean negative control OD value is ＜ 0.05, calculated by actual value when Mean negative control OD value is ＞ 0.05)
Positive Results: Sample OD value ≥ C.O.
Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that hepatitis B virus surface antigen has probably been detected using HBsAg ELISA. All initially reactive specimens should be retested in duplicates using HBsAg ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for hepatitis B virus surface antigen with HBsAg ELISA.
Negative Results: Sample OD value < C.O.
Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no hepatitis B virus surface antigen has been detected with HBsAg ELISA, therefore the patient is probably not infected with HBV and the blood unit do not contain hepatitis B virus surface antigen and could be transfused in the case that other infectious diseases markers are also absent.

REFERENCES

1. Pharmacopoeia of the People's Republic of China.
2. Requirements for Biologics of the People's Republic of China.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

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