



Hepatitis B Virus Surface Antigen (HBsAg) ELISA Test Kit

NAME AND INTENDED USE

Diagnostic Kit for Hepatitis B Virus Surface Antigen (HBsAg) (ELISA). It is used in qualitative tests for HBsAg. This reagent may be directly used with human serum for detecting HBsAg.

CLINICAL INTEREST

The detection of HBsAg in the serum or plasma indicates an infection caused by the Hepatitis B virus. It is the first marker to appear and may be observed 2 or 3 weeks before the clinical and biological symptoms of the disease. Its period of presence may be very short (a few days) or very long (several years). Beyond 6 months of persistence of HBsAg, Hepatitis is called "chronic".

Because of the existence of numerous asymptomatic chronic carriers, Hepatitis B represents an important transfusion hazard and the prevention of the transmission is based upon the detection of the HBsAg at time of each blood donation.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

HBsAg uses a "sandwich principle", Enzyme-linked immunological sorbent assay. To measure HBsAg levels in serum or plasma, plastic wells coated with two monoclonal antibodies of HBsAg are supplied in the kit. The patient's specimen is added and, during incubation, HBsAg, if present, is fixed to the solid phase antibody, when antibody of HBsAg (polyclonal) with HRP is added, it binds to HBsAg on the well, creating a HRP-antibody-HBsAg-antibody "sandwich". After the added of TMB substrate, the result is visualized by the naked eye or obtained by EIA plate reader.

PRECAUTION FOR USERS

1. This kit contains human sourced components. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Handling should preclude any pipetting by mouth.
3. There should be no smoking or eating where infections containing materials are being handled.
4. Hands should be covered with rubber gloves during and thoroughly washed after handling of potentially infectious materials.
5. The specimens found to be reactive by test and all materials used to perform the tests should be handled and disposed of as if they contained the infectious agent of viral hepatitis. The preferred method of disposal is auto-claving for a minimum of one hour at 121.5°C. Rubber gloves worn throughout the entire procedure should also be decontaminated before discarding. Disposable materials may be incinerated. Liquid waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 2.5% sodium hypochlorite. Allow 30 minutes for sterilization.
6. To avoid microbial contamination of reagents, aseptic techniques should be used in removal of aliquots from the primary vials.
7. Use only pipettes with disposable tips for each specimen.
8. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by HBsAg procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time can be frozen at -20°C and avoid repeated freeze thaw.

NOTE: If need, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8x12 wells), Ready to use. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2-8°C until expiration date.
2. HRP Conjugate: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
3. Positive control: 1 vial of 1ml, Ready to use. Store at 2-8°C until expiration date.
4. Negative control: 1 vial of 1ml, Ready to use. Store at 2-8°C until expiration date.
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
8. Wash buffer: 1 vial of 30ml, diluted 1:20 before use. Store at 2-8°C until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

RELATED TIPS

1. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 5 times with about 0.3ml diluted washing solution. If no automatic
2. Washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with diluted wash solution and remain 20 seconds. Repeat these steps 5 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are
5. used, there is no need to correct the zero point.
6. Storage. The whole kit should be stored at 2-8°C for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash Solution by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. Set one blank well for background, two wells for negative control, two wells for positive control and other wells for samples.
2. Dispense 100 ul of Negative control as well as Positive control or samples into respective wells.
3. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hour.
4. Remove the coated plate, without washing, directly in each hole to join the enzyme conjugate 50ul (except blank well).
5. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.
6. Wash each well for 5 times, 20 seconds each time.
7. Dispense 50 ul (or one drip) of chromogen A to each well (including the blank well).

8. Dispense 50 ul (or one drip) of chromogen B to each well (including the blank well).
9. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.
10. Dispense 50 ul (or one drip) of stop solution to each well (including the blank well) and mix completely.
11. Read the absorbance of the plate within 10 minutes. (See read procedure)

DETECTION RESULTS OF THE TEST

1. Calculation mean value of the OD of Negative control wells(N). If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.
2. Calculation of the cutoff value.
Cutoff value = $2.1 \times N$
3. The specimen is positive if: OD value \geq cutoff value.
The specimen is negative if: OD value $<$ cutoff value.

PERFORMANCE CHARACTERISTICS

1. Sensitivity
adr $\leq 0.11U/ml$ adw $\leq 0.11U/ml$ ay $\leq 0.21U/ml$
2. Precision
Interassay $\leq 15\%$
Intraassay $\leq 10\%$