SPECTRUM

The Creative Approach to Bioscience

HBsAg ELISA

REF: 1304 001 96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the qualitative detection of HBsAg (hepatitis B Surface Antigen) in human serum or plasma.

Background

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (HBV). This infection of the liver is transmitted by homosexual or heterosexual activity, blood borne exposure, mother - infant. In the HBV infected people, the virus persists for the rest of their lives and can be passed on to others. Therefore Hepatitis B has become a global public health problem. Infection with HBV results in the appearance of a number of serological markers and one of the first of such markers is Hepatitis B surface antigen (HBsAg). The HBV infection causes a wide variety of liver damages such as acute self-limiting infection fulminating hepatitis, chronic hepatitis with progression to cirrhosis and liver failure, and asymptomatic chronic carrier state.Hepatitis B surface antigen (HBsAg) appears 1-7 weeks before biochemical evidence of liver disease or jaundice. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is risk group population.

Assay Principle

This assay is based upon the one-step sandwich Technique. Sample, Anti-HBs coated microplate and enzyme-labeled Anti-HBs are combined. During the incubation, HBsAg present in the sample is allowed to react simultaneously with the two antibodies, resulting in the HBsAg being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the HBsAg within the sample and antibody in enzyme conjugate by immunological reactions. Substrate A and substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is proportional to the amount of HBsAg in the sample.

Reagents

Coated Wells
1 x 96 wells pre-coated with mouse monnoclonal antiHBs.

Negative Control
1 x 1 ml of phosphate buffered solution containing proteins of bovine origin. 0.1% preservative was added.

Positive Control
1 x 1 ml of phosphate buffered solution containing heat-inactivated human plasma positive for HBsAg and proteins of bovine origin. 0.1% preservative was added.

Enzyme Conjugate
1 x 7.5 ml of HRP (horseradish peroxidase) labeled sheep polyclonal Anti-HBs in a buffer containing BSA (bovine serum albumin). 0.1% preservative was added.

Substrate A
1 x 7.5 ml of hydrogen peroxide.
Substrate B
1 x 7.5 ml of TMB (3, 3', 5, 5'-tetramethylbenzidine) in a buffer solution.

Wash Solution Concentrate
1 x 30 ml of 20 times working wash solution.

Stop Solution
1 x 7.5 ml of 0.62 mol/l sulfuric acid.

2 Pieces of plate lid and 1 Zip-lock bag.

Reagents Storage and Stability

1. Store all components at 2-8^{O}C. Do not freeze. Avoid strong light.
2. Store unused wells in a sealed zip-lock bag (with desiccant provided) in the aluminum foiled pouch with a plate, as well as the unused reagents at 2-8^{O}C under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Reagent Preparation

1. Bring all reagents to room temperature (18-25 ^{O}C) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
2. Adjust the incubator to 37 ^{O}C.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled water and mix well. Prepare the required volume according to the number of assayed samples.

Specimen Collection and Preservation

1. Serum and plasma are suitable for analysis. The only acceptable anticoagulants are heparin, EDTA and sodium citrate. Specimen should be promptly separated from cells after blood collection.
2. Cap and store the samples at 18-25 ^{O}C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ^{O}C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ^{O}C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. After thawing, bring to room temperature and mix well by gently shaking.

Precautions and warnings

1. The controls contain human sourced components, which have been tested and found non-reactive for HIV 1 and HIV 2 antibodies, Anti-HTLV I & II and HCV antibodies, in addition, the negative has been tested and found non-reactive for HBsAg, while the positive control has been tested and found reactive for HBsAg. It is recommended that all human sourced materials be considered potentially infectious.
2. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
3. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
4. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
5. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
6. Do not touch or splash the rim of the well with conjugate.
7. Do not mix or use components from kits with different batch codes.
8. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.
9. It is important that the time of reaction in each well is held constant to achieve reproducible results.
10. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and Stop Solution should be added in the same sequence to eliminate any time deviation during reaction.
11. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
12. Do not allow wells to become dry during the assay procedure.

Assay Procedure

1. Determine the total number of wells needed for the assay. In addition to specimens, one substrate blank, three negative controls and two positive controls must be included on each plate or partial plate. Unused wells should be stored in the supplied Zip-lock bag with desiccant provided.
2. Prepare a record (plate map) identifying the placement of the blank, controls and specimens in the microwells.
3. To the wells of controls add 50 µl of the negative or positive controls.

4. Add 50 µl of sample to each of the rest of the wells.
5. Shake on a plate shaker for 30 seconds to completely mix the samples the wells.
6. Add 50 µl of enzyme conjugate to each well except the blank well
7. Shake on a plate shaker for 30 seconds to completely mix the liquid within the wells.
8. Cover the plate with a lid and incubate at 37 ^{O}C for 30 minutes.

9. Add 350 µl of wash solution, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
10. Add 50 µl of substrate A, then 50 µl of substrate B to each well, including the blank well.
11. Gently mix for 15 seconds and incubate at 37 ^{O}C in the dark for 10 minutes without shaking.
12. Add 50 µl of stop solution to each well, including the blank well and mix gently.
13. Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize interference) in a microplate reader. 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.

Assay Scheme	
Controls / samples	50 µl
Enzyme Conjugate	50 µl
Incubation time	30 minutes
Washing step	6 times
Substrate A Substrate B	50 µl 50 µl
Incubation time in dark	10 minutes
Stop solution	50 µl
Read absorbance	450 nm

Test Validation

The recommended control requirement for this assay is using positive and negative controls to verify assay performance. The result is valid if the following criteria for the controls are both met

Positive Control

Mean absorbance of Positive Control is equal to or higher than 0.6

Negative Control

Mean absorbance of Negative Control is lower than 0.1

Results

Each plate must be considered separately when calculating and interpreting results of the assay.

Cut-off Value

Calculate the mean absorbance of the replicates of the Negative Control.

The cut-off = 2.1 X Mean of the Negative Control replicates

N.B. If the mean absorbance of Negative Control replicates < 0.05, use the following equation:

The cut-off = 2.1 X 0.05

Example

Mean Negative Control	= (0.021 + 0.017+ 0.013)/3 = 0.017
Cut-off Value	= 2.1 × 0.05 = 0.105

Interpretation of Result

Nonreactive Results
Samples giving an absorbance less than the cut-off value are considered nonreactive.

Reactive Results
Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay. Such samples should be retested in duplicate . Samples that are reactive in at least one of the retests are considered positive for HBsAg and should be confirmed by using a confirmatory kit. Samples that are nonreactive in both wells on retest should be considered nonreactive.

Limitations
1. This assay is intended as an aid for the clinical diagnosis Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results.Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.

Diagnostic Sensitivity
The diagnostic sensitivity was determined by testing a panel of 400 positive samples. The results obtained show a diagnostic sensitivity of 100 %.

Diagnostic Specificity
The diagnostic specificity was determined by testing a panel of 10040 samples, in parallel with a test already available on the market. The results obtained show a diagnostic specificity of 99.5%.

Performance Charateristics

Measurement pricision

Within run (Repeatability)

With one human serum based panel, using one batch of reagent in replicates of 100 samples data are as following:

Mean = 1.18 **SD =** 0.072 **CV =** 5.62 %

Run to run (Reproducibility)

With one human serum based panel, using one batch of reagent in replicates of 100 samples across 3 separate runs data are as following:

Mean = 1.14 **SD =** 0.088 **CV =** 7.43 %

References

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