Website : www.erbamannheim.com

ERBA LISA PICO HBsAg

ERBA LISA PICO HBsAg is an immunoassay for the detection of Picogram to Nanogram Quantity of HBsAg in human Serum & Plasma.

INTRODUCTION

Hepatitis B Virus (HBV) is responsible for both Hepatitis B and hepatocellular carcinoma. HBV affects about 5% of world population. One out of every ten persons infected with Hepatitis B infection develops some form of chronic liver disease and becomes a long term carrier of HBV. At various stages after HBV infection, presence of Hepatitis B surface antigen (HBsAg) in serum is identified. HBsAg is a set of lipoprotein, molecular weights ranging from 22 kD to 96 kD, constitutes the envelope of the virus. HBsAg is the first detectable marker in HBV infected serum and being detectable during the whole jaundice phase and becomes undetectable after the appearance of anti-HBsAg in serum.

ERBA LISA PICO HBsAg uses polyclonal antibodies to HBsAg as coating materials and monoclonal antibodies to HBsAg as conjugate materials.

PRINCIPLE

The **ERBA LISA PICO HBsAg** test kit is a solid phase immunoassay for the qualitative detection of HBsAg in human serum and plasma.

Addition of positive control or HBsAg containing human serum or plasma will form a stable complex with the bound antibody present in the well and with anti-HBsAg-HRPO. A washing step will remove the unbound conjugate molecule. Addition of color reagent will develop blue color only in positive control wells and wells containing HBsAg in test specimen. Upon addition of stopping solution, blue color changes to yellow. The intensity of developed yellow color is directly proportional to the presence of bound HBsAg in the respective wells.

CONTENTS OF THE KIT

	96 Test	192 Test
1. Anti-HBsAg coated Plate	1	2
2. Conjugate	6.0ml	12.0ml
3. HBsAg Positive Control	1.5ml	2.0ml
4. HBsAg Negative Control	1.5ml	2.0ml
5. Color Reagent	6.0ml	12.0ml
6. Sample Diluent P	3.0ml	6.0ml
7. Stopping Solution	12.0ml	24.0ml
8. Washing Solution (20×)	20.0ml	40.0ml
9. Black Cover	1	2
10. Adhesive Strips	2	4

Items 1 to 8 should be stored at 2-8°C and rest items i.e., 9 & 10 can be stored at room temperature (20-30°C).

MATERIALS REQUIRED BUT NOT PROVIDED

1. 0-20 µl and 50-200 µl micropipettes and disposable tips.
2. Automatic micro plate washing instrument.
3. Precision ELISA reader.
4. Disposable gloves.
5. Timer.
6. Measuring cylinder – 500.0 ml.

STORAGE

1. The kit should be stored at 2-8°C. **Do not FREEZE.**
2. Immediately after use, return the reagents at 2-8°C.

PRECAUTIONS

1. Disposable gloves should be worn throughout the procedure.
2. For ***in vitro*** diagnostic use only.
3. The positive control serum has been inactivated. This does not ensure the complete absence of viable HBV, and therefore, this serum should be treated as infectious materials.
4. Prior to disposal, all waste materials should be collected and kept in 5% sodium hypochlorite solution for 30 minutes.
5. Do not use the kit beyond its labeled expiry date.
6. Do not interchange reagents between different lots.
7. Use clear serum. Particulate matter should be removed by centrifugation.
8. Use separate tips for controls and individual test specimens.
9. Do not expose color reagent to sunlight.
10. Distilled or deionised water to be used for dilution of washing buffer.
11. **After using required number of Strips, rest of the strips along with activated silica gel should be kept in sealed condition in to the polythene zip lock bag.**
12. **Do not use remaining kit after four weeks once open the kit.**

SPECIMEN COLLECTION AND STORAGE

1. Handle all test specimens as potentially biohazardous.
2. Early separation of serum from the clot prevents haemolysis of serum. Specimen should be collected aseptically.
3. Undiluted serum should be stored at 2-8°C.
4. Frozen specimen must be thawed properly.

MICROPLATE WASHING PROCEDURE

Dilute washing solution (1+19) in distilled or deionised water. Washing solution may be crystallized at cool storage condition. If so, use it after thawing at 37°C water bath. We suggest that 6 cycles with at least 0.35 ml wash buffer per well per wash and a soak time of 30 seconds. **Invert the plate and tap it on absorbent pad to remove the remaining washing solution.**

Manufactured by : **TRANSASIA BIO-MEDICALS LTD.,** B-11, O.I.D.C., RINGANWADA, DAMAN-396 210 (INDIA).
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TEST PROCEDURE:

1. Bring all the reagents and test specimens at room temperature before use.

2. Add 25 µl of sample diluent to each well. In each run, there will be one blank (100 µl sample diluent plus 50 µl conjugate), three negative controls and one positive control. Add 75 µl of control and test specimens to the respective wells. Add 50 µl of conjugate to each well. **Cover the plate with black cover and incubate 60 minutes at 37°C.**

3. Wash the plate as per micro plate washing procedure.

4. Add 50 µl of color reagent. Cover the plate with black cover and incubate for 15 minutes in dark at 20-30°C.

5. Add 100 µl of stopping buffer to each well.

6. Read absorbency at 450 nm (using 620/630/650 nm as reference wavelength). Deduct blank absorbency from the control and test wells.

CALCULATION FOR CUT-OFF VALUE

Blank value: Absorbency of blank value should be less than 0.05

Positive Control: Absorbency of individual positive control should be greater than 1.0.

Negative Control: Absorbency of individual negative control should be less than **0.02 after deducting the blank absorbency.**

Note:
NCx: Average value of negative controls.

Calculation of NCx:
Example:

NC	Absorbance
1	0.006
2	0.005
3	0.007

NCx: (0.006+0.005+0.007)/3 = 0.006

Cut-Off Value Formula: 0.05 + NCx

Cut-0ff value: 0.05 + 0.006 = 0.056

Note:
The OD of clear samples (NC, Negative Human Serum or Normal Human serum) can be in minus. However the minus (-) O.D does not in any way affect the result interpretations. It rather gives the better specificity.

Interpretation of the result:

Non-Reactive: If the absorbency of the test serum is less than the cut-off value, then the sample is considered as **non-reactive**.

Reactive: If the absorbency of the test serum is equal or greater than the cut-off value, then it is considered as **initial reactive.** This sample should be retested as duplicate. If the absorbency of duplicate retest results are less than cut-off value, then the specimen is considered as **non-reactive.** If both of duplicate retest results are found reactive, then the specimen is considered as **repeatedly reactive.**

SENSITIVITY: The relative sensitivity of **ERBA LISA PICO HBsAg** was compared by using 30 spiked panel of HBsAg. Each panel was assayed in replicate of 4 for 6 days. The mean sensitivity **was less than 50 pg/ml.**

BIBLIOGRAPHY

1. Engvall E *et al* (1971). Enzyme-linked Immunosorbent assay II. Quantitative assay of Protein antigen, Immunoglobulin G, by means of enzyme-linked antigen and antibody-coated tubes. Biochem. Biophys Acta, 251: 427-434.

2. Wolters G *et al* (1976). A solid phase enzyme-immunoassay for detection of Hepatitis B surface antigen. J. Clin. Pathol. 29: 873-879.

3. David G.S *et al* (1981). Monoclonal antibodies in the detection of hepatitis infection. Med. Lab. Sci. 38: 341-348.

4. Fields H.A *et al* (1983). Experimental conditions affecting the sensitivity of Enzyme Linked Immunoabsorbent Assay for detection of Hepatitis B surface antigen (HBsAg). Bull. W. H. O, 61: 135-142.

5. Jongerius J.M *et al* (1998). New Hepatitis B Virus mutant form in blood donor that is undetectable in several Hepatitis B surface antigen screening assays. Transfusion, 38: 58-59.

6. Locamini S.A *et al* (1998). Hepatitis B surface antigen and Polymerase gene variants: Potential Virological & Clinical Significance. Hepatology, 27: 294-297.

7. Hunt C.M *et al.* (2000). Clinical Relevance of Hepatitis B viral mutations. Hepatology, 31: 1037-1044.

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