

BHAT BIO-TECH INDIA (P) LTD.

CE 1023 **HEPA-SCAN®**
HBsAg ELISA TEST

A solid-phase enzyme immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma

For Professional Use IVD

READ THIS PACK INSERT CAREFULLY BEFORE PERFORMING THE TEST

Structure of Hepatitis B virus

CLINICAL SIGNIFICANCE :
In most cases of Hepatitis B infection incubation period varies from 40 days to 6 months, but is around two to three months on an average. A prodromal illness occurs in some patients who complain of malaise and anorexia accompanied by weakness and myalgia. Anthralgia also occurs and may be accompanied by an urticarial or maculopapular rash. The diagnosis of Hepatitis B infection depends on finding components of virus and specific antibodies in the blood.

Schematic Representation of "Hepa-Scan" HBsAg ELISA

STORAGE AND STABILITY :
Storage :
Store the kit between 2-8°C. DO NOT FREEZE. The bag containing microtiter plate must be brought to Room temperature (20-30°C) before opening, to avoid condensation in the wells. Unused wells should be sealed in the bag, and refrigerated (2-8°C). After opening the sealed pouch, unused strips are stable for 2 months at 2-8°C in the original pack sealed with tape. Do not return the holder to the pack.

Stability :

- The unopened kit is stable for 1 year from the date of manufacturing as indicated on the package.
- The opened kit is stable for 3 months from the date of opening.
- Repeated freeze thaw of reagents from 2-8°C to Room temperature several times will reduce the stability of the kit.

PACK SIZE : Available in packs of 96 Tests and 48 Tests.

CONTENTS OF THE KITS :

| Materials | 96 Tests | 48 Tests |
|---|----------|----------|
| Anti-HBsAg coated microwells (12x6 wells strip) | 62 wells | 31 wells |
| Wash solution (Concentrated 10X) | 50ml | 25ml |
| Anti-HBs HRP Conjugate (Ready to use) | 3 ml | 1.5 ml |
| TMB Substrate (Ready to use) | 11 ml | 6 ml |
| Stop solution (Ready to use) | 12 ml | 6 ml |
| Positive control | 1 ml | 0.5 ml |
| Negative control | 1 ml | 0.5 ml |
| Adhesive slips | 2 Nos. | 1 No. |

PRECAUTIONS :

- For in vitro diagnostic use only.
- The positive control contains inactivated Hepatitis B virus. However, it should be treated as infectious. The Negative serum also should be treated as infectious.
- All human serum and plasma samples should be considered potentially infectious. It is recommended that all specimens of human origin should be handled as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease control/National Institute of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.
- Never pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable latex gloves while handling specimens and kit reagents. Afterwards wash hands carefully with disinfectants. Avoid splashing or forming aerosols.
- Discard all materials and specimens capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C.
- Liquid wastes not containing acid may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500mg/dl available chlorine. Allow 30 minutes for decontamination to be completed.

NOTE :

- Liquid wastes containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
- Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.

INDICATIONS OF INSTABILITY AND DETERIORATION OF REAGENTS :

- Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use reagents, which are visibly turbid.
- The TMB SUBSTRATE solution should be colorless for proper performance of the assay. Any color may indicate deterioration of the TMB substrate.

TEST PROCEDURE :

Preliminary preparations

- Wear disposable latex gloves throughout the procedure.
- Bring all reagents and Micro wells to Room temperature (25-30°C) before starting the assay. Gently mix all liquid reagents before use.
- Dilute the wash solution 1/10th with distilled or deionised water. Diluted wash solution should be stored at 2-8°C and is stable for 2 weeks. If the concentrated solution shows any crystals, dissolve them by warming in a water bath at 37°C before dilution.

TEST PROCEDURE :

- Set up microtiteration wells in the frame provided.
- Label 3 wells as positive and 3 wells as negative control.
- Add 50ul of control or test sample to approximately labeled wells of the microtiter plate.
- Add 25 ul of Anti-HBs HRP conjugate solution to each well and mix thoroughly by gentle swirling.
- Cover the wells with adhesive slips.
- Incubate at 37°C for 90 minutes.

In the above sample, all negative control values are within quality control range and the NCx need not be revised. **CALCULATION OF THE POSITIVE CONTROL MEAN (Pcx)**
Determine the mean of the positive control values.

Example:

| Positive control Sample No. | Absorbance |
|-----------------------------|--------------|
| 1 | 2.523 |
| 2 | 2.505 |
| 3 | 2.490 |
| Total | 7.518 |

$Pcx = \frac{\text{Total absorbance} \times 3}{3} = \frac{7.518 \times 3}{3} = 2.506$

In the above example Pcx minus NCx is greater than 0.900, thus the technique is acceptable and data should be considered valid.

CALCULATION OF THE CUT-OFF VALUE (CO)
Determine the cut-off value by adding 0.05 to the negative control mean (NCx). This cut-off value is used to achieve the highest possible sensitivity eg. in blood donor centers.
 $CO = NCx + 0.05$
Example : $CO = 0.098 + 0.05 = 0.148$

RESULTS :

- Non-Reactive :**
A test sample is considered to be non-reactive for HBsAg if the resulting absorbance value is less than the cut-off value.

TROUBLE SHOOTING : BLANK HAS TOO HIGH ABSORBANCE VALUES

| Cause/Error | Remedy |
|--|-----------------------------------|
| 1. Substrate solution is contaminated | Use fresh pipette tips every time |
| 2. Contamination, spills from other wells | Avoid contamination |
| 3. Washing solution has not been diluted correctly | Should be diluted 1/10 (1+9) |
| 4. Poor washing | Check your washer |

POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUE

| Cause/Error | Remedy |
|--|---|
| 1. Substrate solution is contaminated | Use fresh pipette tips every time |
| 2. Interchange of controls from different lots | Do not mix or interchange reagents from different lots. |
| 3. The pipetted volume is too high | Volume should be 50 l |

POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES

| Cause/Error | Remedy |
|--|---|
| 1. Interchange of controls from different lots | Do not mix or interchange reagents from different lots. |
| 2. The pipetted volume is too high | Volume should be as indicated |

| Cause/Error | Remedy |
|--|--|
| 1. Once opened microtiter foil package has not been resealed tightly and stored properly with dessicant. | Once opened microtiter plate foil package has to be resealed tightly and stored properly with dessicant. |
| 2. Interchange of reagents from different lots | Do not mix or interchange reagents from different lots |
| 3. Substrate solution is exposed to direct sunlight. | Avoid unnecessary exposure to light |
| 4. Stop solution has not been mixed properly before measurement. | Mix the plate before measuring |
| 5. Deterioration of reagents | Use aseptic technique. Do not pour used reagent back to vials. |
| 6. Contamination of conjugate by human serum or plasma (usually from samples) | Even one microliter of human serum or plasma is enough to inhibit as much as 1 litre of conjugate. |
| 7. TMB chromogen have been too cold | Never pour used reagent back to vial. Should be 25-30°C |

POOR SPECIFICITY

| Cause/Error | Remedy |
|---|---|
| 1. Washing solution has not been diluted correctly. | Should be 1:10 (1+9) |
| 2. Salt crystals in the washing solution concentrate have not been redissolved before diluting. | Redissolve the crystals before diluting by warming and mixing the concentrate |
| 3. Poor washing | Check your washer |
| 4. Too low positive control value | See positive control has too low absorbance value |

| | Intra-assay Variation | | | Operator-to-Operator Variation | | |
|----|-----------------------|-------|------|--------------------------------|-------|------|
| | Mean (A±50nm) | SD | CV% | Mean (A±50nm) | SD | CV% |
| NC | 0.102 | 0.008 | 7.84 | 0.103 | 0.004 | 3.8 |
| PC | 2.673 | 0.046 | 1.72 | 2.691 | 0.018 | 0.66 |

Inter-assay :
The inter-assay variation of Bhat Biotech's Hepa-Scan® HBsAg ELISA was determined by assaying positive and negative samples in 10 independent test runs. Summary of the results is presented in Table-2.

Table 2 : Summary of the inter-assay variation study of Bhat Biotech's Hepa-Scan® HBsAg ELISA Test.

| Serum sample | Mean (O.D.±450nm) | Standard Deviation (SD) | Coefficient of Variation (%) |
|--------------|-------------------|-------------------------|------------------------------|
| NC | 0.100 | 0.002 | 2.00 |
| PC | 2.678 | 0.037 | 1.38 |

B. Sensitivity :

| No. of Positive Samples tested | No. of Positives by Bhat Biotech's Hepa-Scan® HBsAg ELISA Test | Sensitivity (%) |
|--------------------------------|--|-----------------|
| 99 | 98 | 99 % |

REFERENCES :

- NCCLS Document M29-TZ (1991). Protection of laboratory workers from instrument biohazards. Vol. 1, No 15.
- Blumberg, B.S., Alter, H.J. and Visnich, S.A. "New" Antigen in leukemia sera, J.A.M.A. 191: 541-546, 1965.
- Voller, A., Bartlett, A. and Big well, D.E. Enzyme Immunoassays with Special Reference to ELISA Techniques, J.Clin. Pathol. 32: 507-520, 1978.
- Fields, H.A., Devis, C.L., Bradley, D.W. and Maynard, J.E. Experimental conditions affecting the sensitivity of Enzyme Linked Immunosorbent Assay (ELISA) for detection of Hepatitis B Surface Antigen (HBsAg). Bull. W.H.O. 61: 135-142, 1983.

SUMMARY OF PROCEDURE :

Add 50ul of Test sample or Controls
Add 25ul of conjugate
Incubate 90 minutes at 37°C
Aspirate and wash 5 times with working wash solution
Add 100ul of TMB Substrate solution
Incubate for 10 minutes at room temperature (in a dark place)
Add 100ul of stop solution
Read the absorbance at 450nm

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CATALOGUE No. : HBEL

INTENDED USE : Bhat Biotech's Hepa-Scan HBsAg ELISA Test is an in vitro enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma. The HBsAg ELISA is primarily used for testing blood and organ donors, patients with active viral hepatitis and people at risk for hepatitis B infection.

INTRODUCTION :
Hepatitis B virus is responsible for the most serious form of the hepatitis related diseases. HBV is transmitted through sexual contact, blood and other body fluids, certain blood products and also transmitted from an infected mother to her child during the perinatal period. Hepatitis B Surface Antigen (HBsAg) is usually detectable around two to six weeks in advance of clinical and biochemical evidence of hepatitis and persists throughout the clinical course of the disease. Level of HBsAg is of maximum titration at the height of liver damage. HBsAg is a complex lipid and glycoprotein particle consisting of a group specific determinant 'a' and two pairs of type specific determinants, 'd/y' and 'w/r'. HBsAg can thus be divided into four antigenic subtypes : 'adv', 'adr', 'ayw', and 'ayr'. The subtype 'ayw' is predominant in India.

Hepatitis B Virus (HBV) or Dane particle is a double-stranded virus which is 42nm in diameter with a lipoprotein coat and a nucleocapsid core. The lipoprotein coat is composed of Hepatitis B surface antigen (HBsAg). The nucleocapsid is 27nm in diameter and possesses a distinct antigenic specificity (HBeAg) along with a partially double-stranded DNA molecule and an endogenous polymerase enzyme. Another antigen called "Hepatitis B" e antigen (HBeAg) is a split protein of HBeAg.

All the above antigens and viral DNA polymerase form useful diagnostic markers for HBV. However, HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection due to its utmost appearance almost 2-4 weeks before the ALT level becomes abnormal and 3-5 weeks before the onset of symptoms or jaundice as an early detection system for hepatitis B virus infection.

Serological & Clinical Pattern during acute HBV infection

PRINCIPLE :
The principle of Bhat Biotech's Hepa-Scan® HBsAg ELISA is based on direct, non-competitive solid phase enzyme and TMB as the chromogen. The assay has the following reactions.

- When patients serum containing HBsAg is added, it will combine with the goat Anti-HBsAg attached to the polystyrene surface of the Microwells and simultaneously bind with the horseradish peroxidase conjugated goat Anti-HBsAg.
- Wells are washed and a colourless enzyme substrate (H2O2) and chromogen (TMB, Tetramethylbenzidine) are added. The enzyme acts on substrate/ chromogen producing a blue coloured product.
- The enzyme substrate / chromogen reaction is terminated with acid (H2SO4). The yellow colour intensity is directly related to the concentration of hepatitis B surface antigen in the patient sample.

MATERIALS REQUIRED BUT NOT PROVIDED :

- Distilled or Deionised water, preferably sterile
- Graduated cylinders for reagent dilutions.
- Vials to store the diluted reagents.
- Precision pipettes.
- Paper towels or absorbent paper.
- Timer.
- ELISA Reader.
- ELISA Washer.
- Sodium hypochlorite solution (free available chlorine 50-500mg/dl)
- Disposable latex gloves.

SPECIMEN COLLECTION AND HANDLING :
Specimens must be centrifuged before use (e.g 3000 RPM, 10 minutes), especially citrate plasma specimens will cause false HBsAg reactive results, if not centrifuged properly. Serum and plasma (preferably EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20°C or -70°C). Samples should not be repeatedly frozen and thawed.

Do not use heat inactivated samples. Especially heat inactivated plasma specimens will cause false HBsAg reactive results.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase. Microbially contaminated, grossly haemolysed, icteric or hyperlipemic serum and plasma specimens may give erroneous results.

- Deterioration is indicated by a significant decrease in the absorbance level of positive control.
- Avoid exposure of TMB solution to intense source of light. Oxidizing agents; metallic ions or soap remaining in glassware containers can interfere with the TMB reaction. In order to avoid this problem rinse the glassware thoroughly with 1N acid (HCl or H2SO4) followed by several washes with distilled water before use.
- Reagents are stored between 2-8°C. Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB. Storage of reagents and samples in self-detrusting freezers is not recommended.
- Do not use reagents after expiration date mentioned on the label.
- Do not mix or interchange reagents from different kit or kit lots. Cross contamination of reagents or samples can cause erroneous results.
- Stop solution contains sulphuric acid. Avoid contact with skin and eyes.
- Do not interchange vial caps.
- When removing aliquots from the reagent vials, use aseptic technique to avoid contamination, otherwise incorrect results may occur. Use a new pipette tip for each sample. Optimal results will be obtained by strict adherence to the protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements are essential.
- Once the assay has been started, all steps should be performed without interruption.
- Do not touch the wells or scratch the wells while pipetting.
- Do not let wells dry, once the assay has started.
- Reusable glassware must be disinfected, washed out and rinsed free of detergents.

- Wash the microplate 5 times by adding 300ul (approximately) of working wash solution.
- Add 100ul of reagent to use TMB substrate.
- Incalculate at RT for 30 minutes (250C to 300C)
- Avoid expose to light
- Add 100ul of stop solution or (2 drops) to each well.
- Read the absorbance of 450nm on an ALISA Reader within 30 minutes.

RESULTS :

QUALITY CONTROL VALUES

Test validity :
NEGATIVE CONTROL MEAN (NCx)
Individual negative control values should be less than or equal to 0.200 when the photometer is blanked against air. If one of the values is outside the acceptable range, discard this value and recalculate the mean. If two of the values are out of range, the test should be repeated.

POSITIVE CONTROL MEAN (Pcx)
Pcx value has been defined based on lot to lot follow up. To achieve the expected detection limit the value of Pcx minus NCx should be greater than or equal to 0.9. If not, the technique may be suspected and the assay should be repeated.

CALCULATION OF THE RESULTS
THE NEGATIVE CONTROL MEAN (NCx) determine the mean of the negative control values.

| Sample No. | Absorbance |
|--------------|--------------|
| 1 | 0.098 |
| 2 | 0.101 |
| 3 | 0.095 |
| Total | 0.294 |

$NCx = \frac{\text{Total absorbance} \times 3}{3} = \frac{0.294 \times 3}{3} = 0.098$

- Reactive :**
A test sample is considered to be reactive for HBsAg if the resulting absorbance value is greater than or equal to the cut-off value.

INTERPRETATION OF RESULTS :

- Specimens with absorbance values less than the cut-off value are considered non-reactive by Bhat Biotech's HBsAg ELISA test and may be considered negative for HBsAg. Further testing is not required.
- If the values are 10% less or more than the cutoff value (Border line), then the samples must be retested.
- Specimens with absorbance value greater than or equal to the cut-off value are considered initially reactive by Bhat Biotech's Hepa-Scan® HBsAg ELISA Test. The original sample should be retested in duplicate, before final confirmation.
 - Initially reactive specimens which do not react in either of the duplicate, repeat tests are considered negative for HBsAg. Further testing is not required.
 - Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatedly reactive.
 - As in any diagnostic enzyme immunoassay, there is a possibility that repeatable reactions may occur for the following reasons.
 - A test sample is considered to be reactive for HBsAg if the resulting absorbance value is greater than or equal to the cut-off value.
 - Inadequate washing
 - Contamination of reaction well with HRP conjugate
 - Contamination of substrate solution with conjugate or with oxidizing agents.
 - Cross-contamination of non-reactive specimens by HBsAg.

NEGATIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES

| Cause/Error | Remedy |
|--|--|
| 1. Contamination, spills from other wells. | Avoid contamination or interchange of the vial caps. |

ALL ABSORBANCE VALUES VERY HIGH

| Cause/Error | Remedy |
|---|--|
| 1. Interchange of reagents from different lots. | Do not mix or interchange reagents from different lots. |
| 2. Substrate solution is contaminated | Use clean containers |
| 3. Washing solution concentrate has not been diluted correctly. | Should be diluted 1/10 (1+9) |
| 4. Poor washing | Check your washer |
| 5. Contaminated solution containers | Use clean containers |
| 6. Deterioration of reagents | Use aseptic technique. Do not pour used reagent back to vials. |

ALL ABSORBANCE VALUES VERY LOW

| Cause/Error | Remedy |
|---|--|
| 1. Reagent solutions used after they have expired. | Do not use reagents after the expiration date expired. |
| 2. The reagents have not been warmed up to room temperature | Should be 25-30°C when starting the assay |

POOR SENSITIVITY

| Cause/Error | Remedy |
|--|--|
| 1. Too high positive value | See positive control has too high absorbance value |
| 2. Sample serum or plasma is not mixed properly with sample buffer | While pipetting mix the sample with sample buffer |
| 3. Frozen samples have not been mixed properly after thawing | Mix well before pipetting |
| 4. Stop solution has not been mixed properly before measurement | Mix the plate before measuring |

PERFORMANCE CHARACTERISTICS

Accuracy
Bhat Biotech's Hepa-Scan HBsAg ELISA meets the requirement for the third generation test when tested against the FDA approved kits. Subtype specificity of Bhat Biotech's Hepa-Scan® HBsAg ELISA was tested with the HBsAg subtype panel consisting of Ad/ Ay subtypes: HBsAg positive with Bhat Biotech's Hepa-Scan® HBsAg ELISA Test.

A. Precision
Intra-assay
The intra-assay variation of Bhat Biotech's Hepa-Scan® HBsAg ELISA was determined by testing positive and negative samples containing HBsAg. Operator-to-Operator variation was calculated from the results of Intra-assay variation study performed by three technicians. Summary of the results is as follows. Table : Summary of the Intra-assay variation and Operator-to-Operator variation study of Bhat Biotech's Hepa-Scan® HBsAg ELISA Test.

C. Specificity :

| No. of Negative Samples tested | No. of Negatives by Bhat Biotech's Hepa-Scan® HBsAg ELISA Test | Specificity (%) |
|--------------------------------|--|-----------------|
| 200 | 199 | 99.5 % |

Detects as low as 0.2 ng/ml of HBsAg.

Dilution Series Test

| Samples Dilution | Result OD ±50nm |
|------------------|-----------------|
| NI | 2.533 |
| 1:1000 | 2.624 |
| 1:5000 | 2.634 |
| 1:10,000 | 0.881 |
| 1:20,000 | 0.349 |
| 1:40,000 | 0.257 |

KIT PERFORMANCE :
The kit was validated for quality performance as tested with NABI, Boca Raton, and HBV Seroconversion Panel SB 0409.

| Panel Member | HBsAg Test. (Abbott) | Hepa-Scan HBsAg ELISA |
|--------------|----------------------|-----------------------|
| A | + | + |
| B | + | + |
| C | + | + |
| D | + | + |

EN 980:2008 (E) MEDICAL DEVICES SYMBOL

| | | | | |
|---|------------------------------|----------------------------|-------------------------|------------------|
| Temperature Limitation | Date of Manufacture | In vitro Diagnostic Device | LOT | Batch Code |
| Company name & address | Refer Operating Instructions | Use by | Company | Company Name |
| Authorized Representative in European Community | Do Not Reuse | Sufficient for | KEEP AWAY FROM SUNLIGHT | |
| KEEP DRY | NON-STERILE | CONTROL | NEGATIVE CONTROL | POSITIVE CONTROL |

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