ASTAM DIAGNOSTICS

PRODUCT INSERT

REF HBSE

PRATHAM®
HBsAG ELISA

INTENDED USE

PRATHAM® HBsAG ELISA Test is a solid phase, enzyme linked immunosorbent assay for the detection of hepatitis B surface antigen (HBsAG) in human serum/plasma.

INTRODUCTION

Hepatitis B is a disease caused by viral infection. The route of infection can be improper needle puncture, blood transfusion or even by taking contaminated food or water. Hepatitis B has become a significant problem for public health management. Almost one in every ten adults, who have been infected by Hepatitis B Virus (HBV), develops some form of chronic liver disease and becomes a long-term carrier of HBV. Screening for Hepatitis B is therefore urgently needed. Hepatitis B is an immune disease. Invasion of the human body by HB virus induces autoimmune reactions, which damage the liver. The components of the virus (antigens) and the host responses (antibodies), the so-called immunologic markers have often been used as diagnostic tools. There are six immunologic markers of HBV: HBsAg, HBcAg, HBeAg and their respective antibodies. The HBsAg however is the first marker to appear in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier.

TEST PRINCIPAL

PRATHAM® HBsAG ELISA Test strips are coated with monoclonal anti-HBsAg antibodies. Another polyclonal antibody to HBsAg is conjugated to horseradish peroxidase (HRP). The samples are added in the coated wells and incubated. After washing to remove unreacted and unbound material, polyclonal antibody-HRP conjugate is added. The wells are washed to remove unbound components. Bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450nm with an ELISA reader. The cut-off value is calculated by the given formula and absorbance of all the wells are compared with the cut-off value. Any sample having absorbance more than the cut-off value is considered reactive.

KIT COMPONENTS

Component	Description of Reagent	Presentation
Coated Microwells	Micro wells (12x8) coated with monoclonal anti-HBsAG Antibodies	1
Positive Control	HBsAG Positive and HIV/HCV/ non-reactive Serum dilution in stabilizer solution with preservatives.	1x1.5 ml
Negative Control	Inactivated and stabilized human serum non reactive for HIV-1 and HIV-2, HBsAG and HCV.	1x1.5 ml
Conjugate	Polyclonal anti-HBsAG conjugated to HRP.	1x0.8 ml
Conjugate dilution buffer	Buffered solution containing proteins and preservatives.	1x12 ml
Substrate	Solution containing Tetramethibenzidine (TMB) and hydrogen peroxide. Ready to use.	1x10 ml
Wash buffer	Buffer containing surfactants (40X). To be diluted 40 times with distilled or deionized water.	1x25 ml
Stop solution	Diluted sulphuric acid.	1x10 ml
Plate sealer	Adhesive paper sheet for covering plate on incubation time	2 Nos.
Pack insert	Instruction for use	1 Nos.

MATERIAL REQUIRED BUT NOT PROVIDED

Microplate reader capable of measuring absorbance at 450nm, Pipettes and Pipettes tips, Deionized or distilled water, 1X PBS, Automated microplate washer, ELISA Reader, Disinfectant, Timer, Gloves, Biohazard Waste Container etc.

STORAGE AND STABILITY

PRATHAM® HBsAG ELISA test components are stable up to expiry date indicated on the component label/box label. HBsAG ELISA kit needs to be stored at 2-8ºC.

SPECIMEN COLLECTION AND STORAGE

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard procedure.
3. Specimen should be free of particulate matter and microbial contamination.
4. Preferably use fresh sample. However, specimen can be stored refrigerated for 24 hours. For long storage, freeze at - 20ºC or below. Specimen should not be frozen and thawed repeatedly. Maximum of two freeze/thaw cycles are allowed.
5. Do not use heat inactivated specimen.
6. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
7. No not use turbid, lipaemic, haemolysed, clotted or contaminated specimen.

WARNING AND PRECAUTIONS

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Before use, allow reagents to reach room temperature (+18-30ºC).
- Carefully reconstitute or dilute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the micro plate to dry between the end of the washings operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new pipette tip for each sample.
- Washing the micro plate is a critical step in the procedure: Follow the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
- Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped and blotted on absorbent paper or towels to minimize residual wash buffer.
- Never use the same container to distribute conjugate and development solution.
- Check the pipettes and other equipment for accuracy and correct operations.
- Do not change the essay procedure.

Preparation of Reagents prior to use:
Wash Buffer Preparation: Dilute wash buffer 40 times for example add 5 ml concentrated buffer to 195 ml distilled or deionized water.
Conjugate Preparation: Dilute conjugate 21 times as shown below:

Reagent (Strip)	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate (µl)	50	100	150	200	250	300	350	400	450	500	550	600
Conjugate dilution buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Leave well A1 as substrate control.
6. Add 100µl sample or controls in separate well except well A1.
7. Apply plate sealer and incubate for 60 minutes at 37ºC.
8. Wash each well by filling approximately 350µl diluted wash buffer and aspirating/flicking off six times. Blot dry.

9. Add 100µl diluted conjugate in each well except A1 and incubate for 30 minutes at 37ºC.
10. Wash six times as in step 9 and blot dry.
11. Add 100µl substrate in each well including A1 and incubate at room temperature away from light for 15 minutes.
12. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
13. Blank ELISA reader with well "A-1".
14. Read the absorbance at 450nm with 630nm or above as reference within 30 minutes of stopping the reaction.

Quality Control

Negative Control: The individual absorbance value of negative controls should be less than 0.1.
Positive Control: The individual absorbance value of positive controls should be more than 1.0.
Calculation of Cut of Vlue
The cut off value = 0.1 + NC(Avg)
Set up the cut-off value
Example

NC	**Absorbance**
A1	**0.022**
B1	**0.026**

Avg. of NC= (0.022+0.036)/2= 0.024
cov = 0.1+0.024=0.124

INTERPRETATION OF RESULTS

Negative: Specimen with absorbance value less than or equal to the cut-off value are considered Negative for HBsAG.
Positive: Specimen with absorbance value more than to the cut-off value are considered positive for HBsAG. These specimens should be retested in duplicate. On retesting if the absorbance value of the duplicates is less than cut of value, the specimen is considered as negative. If reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
Repeatedly reactive: If a sample is repeatedly reactive the probability of HBsAG is high, especially with patients at high risk or high absorbance values. Such samples should be retested with other tests and correlated with clinical symptoms.

INTERNAL QUALITY CONTROL

1. Positive and negative controls should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination in observed with the HBsAG Negative Control and agglutination with large aggregates is observed with the HBsAG Positive Control.

PERFORMANCE CHARACTERISTICS

The kit has been evaluated with the 70 known panel of HBsAG positive and 150 negative samples. Following is the in-house evaluation.

Description	Negative	Positive	Total
HBsAG known Negative Specimen	150	0	150
HBsAG known Positive Specimen	0	70	70

Assay Sensitivity : 100%
Assay Specificity : 100%

LIMITATION OF THE TEST

1. **PRATHAM® HBsAG ELISA** is a reliable assay; it should not be sole criterion for diagnosis of HBV infection. Reactive sample should be retested with confirmatory assays like Neutralization assays, HBV DNA by PCR etc.
2. Absence of HBsAG does not indicate that an individual is absolutely free of HBV infection.
3. Since various tests for HBV differ in their performance characteristics and antibody composition, their reactivity patterns may differ.
4. Testing of pooled samples is not recommended.
5. As with all diagnosis tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only ne made by the physician after all clinical and laboratory findings have been evaluated.

DISPOSAL

Consider all test run with human specimen as potentially infectious and discard using standard biosafety practices.

DISCLAIMER:

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

The manufacturer and distributors of this product shall not be liable for any losses, liability, claims, coasts or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative in the use of this product.

REFERENCES

1. Caldwell. C.W., Barrel.J.T. Enzyme immunoassay for hepatitis B and its comparison to other methods. Clinchem Acta. 81:305 (1977).
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3. Magnius L.O., Lindhoilil, A Lunchn, P and Carson. S.A. New antigenantibody system. Clinical significance in long-term, carriers of hepatitis B surface antigen. J. Am. Med. Asso. 231:356-359 (1975).
4. Kim. C.Y. and Tikes. J.G. Purification and biophysical characterization of hepatitis antigen. J. Clin Incest. 52: 1176-1186 (1973) .

SYMBOLS

Read instructions for use	Name of Manufacturer	For single use only
No. of test	Expiry Date of Kit.	Date of manufacturing of IVD Kit
IVD In-vitro diagnostic use	Keep away from Sunlight	REF Reference Catalogue Number
Storage Condition		

ASTAM DIAGNOSTICS

Manufactured By:

ASTAM DIAGNOSTICS PVT. LTD.

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