PATHOZYME® HBsAg Ref OD047 / OD037 / OD207

Enzyme-Immunoassay (EIA) for the detection of Hepatitis B Surface Antigen in human serum.

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro diagnostic use only.

INTRODUCTION

PATHOZYME-HBsAg is an Enzyme-Immunoassay (EIA) for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum.

Acute Viral Hepatitis, caused by the Hepatitis B Virus, is a common and serious infection. It is characterised by inflammation and necrosis of the liver and with the exception of chronic liver disease, it is the single most significant cause of mortality by liver cancer.

Transmission of Hepatitis B Virus occurs by direct human transmission via parental routes such as infective serum, blood, blood transfusion and contaminated needles or by non-parental transmission through body fluids such as saliva, urine and semen.

Tests to detect HBsAg are now widely used for the detection of infected blood products, infected patients and healthy carriers of the disease.

Pathozyme HBsAg is classified as a Third Generation test according to the specifications of the US. F.D.A.

INTENDED USE

PATHOZYME-HBsAg is an in-vitro diagnostic test for the detection of Hepatitis B Virus Surface Antigen in human serum. For professional use only.

PRINCIPLE OF THE TEST

Monoclonal antibodies, specific for the eight known HBsAg subtypes, recognised by the W.H.O., are bound to the surface of microtitration wells. Undiluted test serum are added followed by Anti-HBsAg antibody conjugated to Horseradish Peroxidase (HRP). If HBsAg is present in the sample it binds to the antibody in the wells and the Conjugate binds to the captured viral antigens. If HBsAg is not present binding does not take place and unbound material is washed away. On addition of the Substrate, stabilised 3, 3', 5, 5' Tetramethyl Benzidine (TMB), a colour will develop only in those wells in which the HRP is present, indicating the presence of HBsAg. The reaction is stopped by the addition of dilute Sulphuric Acid and the absorbance is then measured at 450nm. Any result with an optical density (OD) greater than the cut-off should be considered positive.

This test has been calibrated to the International Standard for Hepatitis B Surface Antigen 80/549.

CONTENTS	Ref OD047 (96)	Ref OD037 (192)	Ref OD207 (480)
Microtitre Plate	12x8	12x8x2	12x8x5
Breakable wells coated with monoclonal antibody contained in a resealable bag with a desiccant			
Control - Reagent 2	2ml	2ml	4ml
Negative Control. Clear solution of human serum negative for HBsAg. Ready for use. (Blue)			
Control + Reagent 4	2ml	2ml	4ml
Positive Control Serum. Clear solution of human serum containing HBsAg. Ready for use. (Red)			
Washbuf 10X Reagent 5	50ml	62.5ml x2	62.5ml x4
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)			
Conj Reagent 6	11ml	22ml	27.5mlx2
Anti-HBsAg HRP Anti-HBsAg Conjugated to Horseradish Peroxidase. Ready for use. (Purple)			
Subs TMB Reagent 7	11ml	11mlx2	11mlx5
Substrate Solution: 3,3', 5,5' Trimethyl Benzidine in a citrate buffer.Ready to use. (Colourless)			
Soln Stop H2SO4 0.2M Reagent 8	11ml	11ml x 2	32.5ml x 2
Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)			
Instruction leaflet and EIA Data Recording Sheet	1	1	1
Sealable Polythene Bag: For use during plate incubations, if no humidity chamber is available.	1	2	5

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl, 1000µl and 5000µl
Disposable pipette tips
Incubator: Temperature of 37°C +/- 1°C
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME HBsAg contains materials of human origin. The Negative Control Serum (Reagent 2) has been tested and confirmed negative for HCV, HIV I and II antibodies, and HBsAg by approved procedures. The Positive Control Serum (Reagent 4) has been tested and confirmed negative for HCV, HIV I and II antibodies but positive for HBsAg. The positive control has been heat treated for 10 hours at 65°C to inactivate the Hepatitis B Virus. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use, and for disposal. Do not ingest.

PATHOZYME HBsAg Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME HBsAg Stop Solution (Reagent 8) is 0.2M Sulphuric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with running water.

PATHOZYME HBsAg reagents contain 1.0% Proclin 300™ * as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

*Proclin 300™ is a trade mark of ROHM and HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversly affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Wash Buffer: Dilute the concentrated Wash Buffer (Reagent 5) using 1 part Wash Buffer concentrate with 9 parts distilled water. For every 8 well breakable strip, prepare 25ml of diluted Wash Buffer by adding 2.5ml of concentrated Wash Buffer to 22.5ml of distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

TEST PROCEDURE

The plates provided have eight well strips which may be broken into individual wells if required.

COMBINED PROTOCOL

Sample + Conjugate	Substrate	Total Time
60 mins @ 37°C	30 mins @ 37°C	90 minutes
WASH		

COMBINED ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assy.
2. One set of Control Serum (Reagent 2 & 4) should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 100µl of test serum, Negative Control Serum (Reagent 2) and Positive Control Serum (Reagent 4) into the appropriate wells. Then dispense 100µl of Anti-HBsAg HRP Conjugate (Reagent 6) into each of the wells. Gently shake plate for 60 seconds and place plate into bag or Humidity Chamber. Incubate at 37°C for 60 minutes.
5. Dilute Wash Buffer as described in the Reagent Preparation Section.
6. At the end of the incubation period, remove the plate from the incubator. Wash plate five times with the diluted Wash Buffer with a **60 second soak during each cycle.** Ensure that 300µl is dispensed per well and that an appropriate disinfectant is added to the waste collection container. Remove excess fluid by tapping the inverted plate on absorbent paper.
7. Dispense 100µl of Substrate Solution (Reagent 7) into each well. Gently shake plate for 5 seconds.
8. Allow the reaction to develop in the dark at 37°C for 30 minutes.
9. Stop the reaction by adding 100µl of Stop Solution (Reagent 8) into each well. This will produce a colour change from blue to yellow in wells containing enzyme which indicates the presence of HBsAg. Blank the plate reader on air. Measure the absorbance of each well at 450nm IMMEDIATELY after stopping the reaction.

SEQUENTIAL PROTOCOL

Sample	Conjugate	Substrate	Total Time
60mins @ 37°C	30mins @ 37°C	30mins @ 37°C	120 minutes
WASH	WASH		

SEQUENTIAL ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assy.
2. One set of Test Serum should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 100µl of test serum, Negative Control Serum (Reagent 2) and 50µl of the Positive Control Serum (Reagent 4) into the appropriate wells. Gently shake plate for 5 seconds and place plate into bag or Humidity Chamber. Incubate at 37°C for 60 minutes.
5. Dilute Wash Buffer as described in the Reagent Preparation Section.
6. At the end of the incubation period, remove the plate from the incubator. Wash plate five times using the diluted Wash Buffer with a **60 second soak during each cycle**. Ensure that 300µl is dispensed per well and that an appropriate disinfectant is added to the waste collection container. Remove excess fluid by tapping the inverted plate on absorbent paper.
7. Dispense 100µl of Anti-HBsAg HRP Conjugate (Reagent 6) into each well. Gently shake plate for 5 seconds and place plate into bag or Humidity Chamber. Incubate at 37°C for 30 minutes.
8. Wash plates as described in step 5.
9. Dispense 100µl of Substrate Solution (Reagent 7) into each well. Gently shake for 5 seconds.
10. Allow the reaction to develop in the dark at 37°C for 30 minutes.
11. Stop the reaction by adding 100µl of Stop Solution (Reagent 8) into each well. This will produce a colour change from blue to yellow in wells containing enzyme which indicates the presence of HBsAg. Blank the plate reader on air. Measure the absorbance of each well at 450nm IMMEDIATELY after stopping the reaction.

READING OF RESULTS

The plate reader should be set at a wavelength of 450nm, or at 450nm with a 630 reference filter, and blanked on air. Determine the absorbances of each of the specimens and controls.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

CALCULATION AND INTERPRETATION OF RESULTS

For each test and Control Serum determine the Optical Density (OD) obtained in the wells.

Cut off level **= Average OD of Negative Control** (Reagent 2) **+ 0.10**

Assay Validation**:** The OD of the Positive Control (Reagent 4) must exceed 0.300
The OD of the Negative Control (Reagent 2) must be lower than 0.15.

Negative Result: A negative result should have an OD less than the cut-off.
However, results with an OD up to 10% lower than the cut-off value should be considered as equivocal. These samples should be tested again and if the same result is obtained, another assay should be performed after 1-2 weeks. Possible causes for these equivocal results could be contaminated samples, non-specific reactions or samples with HBsAg levels below the cut-off value of the test.

Positive Result: A positive result should have an OD equal or higher than the cut-off value. Positive results should be assayed again with **PATHOZYME HBsAg** and if the sample continues to be positive, a neutralisation confirmatory test should be performed on that sample.

If levels of controls or users known samples do not give expected results, test results must be considered invalid.

EVALUATION DATA

The Antibodies used in this kit have been selected to provide a system which will detect small quantities of HBsAg including all eight subtypes of Hepatitis B Virus. Lowering the cut-off value increases the sensitivity but at the expense of specificity with the number of false positives being increased. A factor of 0.1 is used here but it may be preferable to determine the appropriate cut-off factor for the patient population under test.

COMBINED Assay Sensitivity approximately 0.5ng/ml

With a cut-off of negative control + 0.1

SEQUENTIAL Assay Sensitivity approximately 0.25ng/ml

With a cut-off of negative control + 0.1

REFERENCES

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COMBINED QUICK REFERENCE TEST PROCEDURE

1. Dispense 100µl test sera and ontrol Serum (Reagents 2 & 4). Then dispense 100µl of Conjugate (Reagent 6) into each well. Gently shake for 60 seconds.
2. Incubate for 60 minutes at 37°C.
3. Discard well contents and wash five times with **a 60 second soak during each cycle.**
4. Dispense 100µl of Substrate Solution (Reagent 7) into each well and gently shake for 5 seconds.
5. Incubate in the dark for 30 minutes at 37°C.
6. Dispense 100µl of Stop Solution (Reagent 8) into each well.
7. Read the OD using an EIA reader with a 450nm filter.
8. Interpret results as described in the Interpretation of Results section.

SEQUENTIAL QUICK REFERENCE TEST PROCEDURE

1. Dispense 100µl test serum and Negative Control Serum (Reagent 2) into appropriate wells. Dispense 50µl Positive Control Serum (Reagent 4) into appropriate wells. Gently shake for 5 seconds.
2. Incubate for 60 minutes at 37°C.
3. Discard well contents and wash five times with a **60 second soak during each cycle.**
4. Dispense 100µl of Conjugate Anti-HBsAg HRP (Reagent 6) into each well and gently shake for 5 seconds.
5. Incubate for 30 minutes at 37°C.
6. Discard well contents and wash five times with a 60 second soak during each cycle.
7. Dispense 100µl of Substrate Solution (Reagent 7) into each well. Gently shake for 5 seconds.
8. Incubate in the dark for 30 minutes at 37°C.
9. Dispense 100µl of Stop Solution (Reagent 8) into each well.
10. Read the OD using an EIA reader with a 450nm filter.
11. Interpret results as described in the Interpretation of Results section.

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