

# PATHOZYME<sup>®</sup> DENGUE M Ref OD227

Enzyme-Immunoassay (EIA) for the detection of IgM antibodies to

Dengue in human serum

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro use only.

## INTRODUCTION

PATHOZYME DENGUE kits are indirect enzyme-immunoassays (EIA) for the detection of IgG or IgM antibodies to Dengue, in human sera. Dengue fever is caused by a virus which has four serotypes (DEN 1, DEN 2, DEN 3, DEN 4). The virus has been reported in over a hundred countries and threatens two fifths of the world's population. If left untreated and undetected the more severe forms, Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS) can develop. With DHF and DSS the mortality rate can be as high as 15%. Children under 15 years old are most at risk. Transmission occurs through the bite of infected female *Aedes aegypti* mosquitoes in tropical and subtropical countries and lately an increase has been seen in Western travellers who visit these places. Life long immunity is seen with each subtype, and infection of all 4 subtypes can be found within one individual's lifetime. A secondary infection increases the risk of developing DHF and DSS. Dengue fever is characterised by fever, headache, rash, nausea and vomiting for 4 to 6 days after infection. In DHF there is leakage of plasma, and a rapid increase in body temperature. Early recognition of plasma leakage and fluid replacement will prevent DSS developing. DSS develops in 10% of DHF cases leading to tissue anoxia, organ failure and death. In primary infections there is a rise in IgM antibodies which are detectable 5 days after the onset of illness, and then gradually decrease after a few months. IgG can only be detected after a few weeks from infection. With secondary infection there are low levels of IgM and very high levels of IgG antibodies detectable after 2 days from onset.

## INTENDED USE

PATHOZYME DENGUE M is an *in-vitro* diagnostic test for screening for Dengue IgM antibodies, in primary and secondary infections caused by all 4 serotypes.

For professional use only.

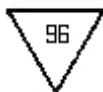
## PRINCIPLE OF THE TEST

Purified Dengue type 2 antigen is coated onto the surface of microtitre wells. In the IgM assay IgG absorb is added to the diluted sera to absorb out the IgG antibodies. IgG absorbed diluted test sera are then applied. Specific antibodies bind to the antigen in the wells. Unbound material is then washed away and peroxidase conjugated anti-human IgM m $\mu$  chain is applied. If antibodies have been bound to the wells, the conjugate will bind to these antibodies.

Unbound material is again 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 enzyme is present indicating the presence of human anti Dengue antibody. The enzyme reaction is then stopped by the addition of dilute Sulphuric Acid and the absorbance is measured at 450nm. Any result with an optical density (OD) greater than the cut off level is considered positive.

This test has been calibrated against in house standards. There is no International standard for this test.

Ref  
OD227



## CONTENTS

<table border="1"><tr><td colspan="2">Microtitre Plate</td></tr></table> Breakable wells coated with specific antigens contained in a resealable foil bag with a desiccant.	Microtitre Plate		12 x 8 wells x 1
Microtitre Plate			
<table border="1"><tr><td>DIL</td><td>SPE</td></tr></table> Serum Diluent: Tris based buffer containing stabilising proteins. Ready to use. (Orange)	DIL	SPE	100 ml
DIL	SPE		
<table border="1"><tr><td>Control</td><td>-</td></tr></table> Negative Control. Clear solution, of human serum negative for IgM antibodies to Dengue. Ready to use. (Blue)	Control	-	2 ml
Control	-		
<table border="1"><tr><td>Control</td><td>L</td></tr></table> Low Positive Control. Clear solution, of human serum containing a low level of IgM antibodies to Dengue. Ready to use. (green)	Control	L	1.25 ml
Control	L		
<table border="1"><tr><td>Sorb</td><td>IgG</td></tr></table> IgG absorb. Goat anti Human IgG. Ready to use. (Colourless)	Sorb	IgG	2.5 ml
Sorb	IgG		
<table border="1"><tr><td>Washbuf</td><td>20X</td></tr></table> Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)	Washbuf	20X	50 ml
Washbuf	20X		

<table border="1"><tr><td>Conj</td></tr></table> Anti-human IgM HRP Conjugate: Anti-human IgM conjugated to Horseradish Peroxidase. Ready to use. (purple)	Conj	5.5 ml			
Conj					
<table border="1"><tr><td>Subs</td><td>TMB</td></tr></table> Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	Subs	TMB	11 ml		
Subs	TMB				
<table border="1"><tr><td>Soln</td><td>Stop</td><td>H2SO4</td><td>0.2M</td></tr></table> Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)	Soln	Stop	H2SO4	0.2M	11 ml
Soln	Stop	H2SO4	0.2M		
Instruction Leaflet and EIA Data Recording Sheet				1 + 1	

## MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100 $\mu$ l, 200 $\mu$ l, 1000 $\mu$ l and 5000 $\mu$ l  
Disposable pipette tips  
Tubes for Sample Dilution  
Vortex Mixer  
Incubator: Temperature of 37°C +/- 1°C  
Absorbent paper  
Microplate reader fitted with a 450nm filter  
Graph paper  
Thoroughly clean laboratory glassware.

## PRECAUTIONS

PATHOZYME-DENGUE M contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and HIV II antibody and HBsAg by approved procedures at single donor level. 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-DENGUE M 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-DENGUE M Stop Solution is 0.2M Sulphuric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with running water.

PATHOZYME-DENGUE M reagents contain 0.05% Proclin 300™ as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water.

\* Proclin 300™ is a trade mark belonging to 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 adversely 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.

## READING OF RESULTS

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

SERUM DILUTION 1/20. This can be achieved by adding 10µl of serum to 200µl of Serum Diluent. Each test uses 35µl of the diluted patient serum.

The plate reader should be set at a wavelength of 450nm and blanked on air. In determining the absorbances of each specimen and control. It is preferable not to use a reference filter as it will change the expected values of the controls.

## 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.

### Wash Buffer:

Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with 19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml 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.

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

## 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 and Epidemiologic factors into consideration. Paired samples taken over a period of a few weeks is recommended. Seroepidemiology may vary between regions and cut off may require adjustment on local studies. Diagnosis should not be made solely on the findings of one assay as early on in infection patients may not have produced detectable levels of antibodies. One assay can not distinguish if the infection is primary or secondary infection.

## 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 assay.
2. One set of control 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.
3. Dilute each test sera 1/20 in Serum Diluent by adding 10µl of serum to 200µl of Serum Diluent. DO NOT DILUTE THE CONTROL SERUM.
4. To 35µl of diluted serum and working strength low positive control add 25µl of IgG absorb. Place in an incubator at 37°C for 15 minutes.
5. Dispense 50µl of diluted serum or low positive control from step 4 and Negative control into the appropriate wells. The control serum should be added last to ensure accurate interpretation of the results. Gently shake for 5 seconds. Cover the plate with the plate lid provided and place it on top of the moist absorbent paper for 37°C for 60 minutes.
6. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
7. Hand Washing: Fill the wells with a minimum of 300µl of wash buffer per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper.
8. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
9. Machine Washing: Ensure that 300µl of wash buffer is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 3 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. Dispense 50µl of Anti-human HRP IgM Conjugate into each well. Gently shake the plate for 5 seconds before replacing the lid onto the plate and returning to the incubator ensuring that the plate is positioned on top of the moist absorbent paper. Incubate at 37°C for 30 minutes.
11. Wash plate as described above.
12. Dispense 100µl of Substrate Solution into each well. Gently shake for 5 seconds before replacing the lid onto the plate and placing in the dark at room temperature (20°C to 30°C) for 15 minutes.
13. Stop the reaction by adding 100µl of Stop Solution to each well. This will produce a colour change from blue to yellow in wells containing enzyme, which indicates the presence of anti-Dengue antibodies. Blank the plate reader on air.
14. Measure the absorbance of each well at 450nm IMMEDIATELY after stopping the reaction.

## 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 OF RESULTS

For each test and Control sera, determine the (OD) obtained in the wells.

Assay Validation: The average OD of the Negative Control should be less than 0.2 the Low Positive Control should be greater than 0.30 for the assay results to be valid.

Cut off level for IgM = Average OD value of the duplicate low Positive Control.

For comparisons between assays antibody indexes (AI) must be calculated:

$$AI = \frac{\text{OD of sample}}{\text{Average OD of Low Positive Control}}$$

AI < 1 is suggestive of no dengue infection. Re-testing is recommended after a few days.

AI > 1 is suggestive of dengue infection. IgM assay is performed to identify acute infections as IgM antibodies rise significantly 3-5 days from fever onset.

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

## EVALUATION DATA

Calibrated to major competitors and in house standards.

The co-efficient of variation of **PATHOZYME PATHOZYME DENGUE M** is less than or equal to 10%.

	Pathozyme Dengue M		Totals
	Positive	Negative	
Positive	52	1	53
Negative	4	34	38
	56	35	91

Sensitivity 52/53 = 98.11%

Specificity 34/38 = 89.47%

## REFERENCES

- (1) Technical advisory group on dengue haemorrhagic fever/dengue shock syndrome (1986). Dengue Haemorrhagic fever diagnosis, treatment and control. Geneva, Switzerland: World Health Organisation.
- (2) **Monath, T. P. and Heinix F. X.** (1996). In B. N. fields, Knipe, D. M. and Howley, P. M. (Eds), Virology, Lippincott-Raven, NY. *Flaviviruses*. 1016-1021.
- (3) **Mikano Y et al.** (1994). Studies on Serological cross-reaction in sequential Flavivirus infections. *Microbiol. Immunol.*, 38(12). 951-955.
- (4) **Clarke, D. H. and Casals, J.** (1958). Techniques for Hemagglutination and hemagglutination inhibition with arthropodborne viruses. *Am. J. Trop. Med. Hyg.* 7:561-573.
- (5) **Sutherst, R. W.** (1993). Arthropods as disease Vectors in a changing environment. In environmental change and human health. CIBA foundation symposia 175: 124-145.
- (6) **Innis, B. L., Nisilak, A., Nimmannitya S. et al.** (1989). An enzyme-linked immunoabsorbent assay to characterise dengue infections where dengue and Japanese encephalitis cocirculate. *Am. J. Trop. Med.* 40:418-427
- (7) **Ruechusatsawat, K. et al.** (1993). Daily observation of antibody levels among dengue patients detected by enzyme-linked immunosorbent assay (ELISA). *Jpn. J. Trop. Med. Hyg.* 22(1):9-12.

## QUICK REFERENCE TEST PROCEDURE

1. Dilute the test sera 1/20 by adding 10µl of serum to 200µl of Serum Diluent.
2. To 35µl of diluted sera and working strength low positive control. add 25µl of IgG absorb. Prepare the low positive control in duplicate. Mix well and leave for 15 minutes at 37°C.
3. Dispense 50µl of negative control, sera and low positive control made up in step 2 to each well. Gently shake for 5 seconds.
4. Incubate for 60 minutes at 37°C.
5. Discard well contents and wash three times.
6. Dispense 50µl of Anti-human HRP IgM conjugate to each well. Gently shake for 5 seconds.
7. Incubate 30 minutes at 37°C.
8. Repeat step 5.
9. Dispense 100µl of substrate to each well. Gently shake for 5 seconds.
10. Incubate in the dark for 15 minutes at room temperature (20°C to 30°C).
11. Dispense 100µl of Stop Solution to each well.
12. Read the OD using an EIA reader with a 450nm filter.
13. Interpret results as described in the Interpretation of Results section.

8076 ISSUE 5 Revised November 2007

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