PATHOZYME®DENGUE G Ref OD217

Enzyme-Immunoassay (EIA) for the detection of IgG 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 worlds 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. Life long immunity is seen with each subtype, and infection of all 4 subtypes can be found within one individuals 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 G is an *in-vitro* diagnostic test for screening for Dengue IgG 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 microtitration wells. Diluted test sera are then applied. Specific antibodies bind to the antigen in the well. Unbound material is again washed away and peroxidase conjugated anti-human IgG 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 then 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.

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96

Microtitre Plate				**12 x 8 wells x 1**

Breakable wells coated with specific antigens contained in a resealable foil bag with a desiccant.

DIL	**SPE**			**100 ml**

Serum Diluent: Tris based buffer containing stabilising proteins. Ready to use. (Orange)

Control	**-**			**2 ml**

Negative Control. Clear solution, of human serum negative for IgG antibodies to Dengue. Ready to use. (Blue)

Control	**L**			**2 ml**

Low Positive Control. Clear solution, of human serum containing a low level of IgG antibodies to Dengue . Ready to use. (green)

Control	**H**			**2 ml**

High Positive Control. Clear solution, of human serum containing a high level of IgG antibodies to Dengue . Ready to use. (Red)

Wasbuf	**20X**			**50 ml**

Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)

Conj				**11 ml**

Anti-human IgG HRP Conjugate: Anti-human IgG conjugated to Horseradish Peroxidase. Ready to use. (purple)

Subs	**TMB**			**11 ml**

Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)

Soln	**Stop**	**H2SO4**	**0.2M**	**11ml**

Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)

Instruction leaflet and EIA Data Recording Sheet **1 + 1**

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl, 1000µl and 5000µl
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 G contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and HIV II antibodies 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 G 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 G 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 G 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 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.

SERUM DILUTION 1/100. Each test uses 100µl at a 1/100 of the patients serum. This can be achieved by adding 10µl of serum to 1000µl of Serum Diluent.
Do not store diluted sera samples, use within eight hours.

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 diuluted 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.

TEST LIMITATIONS

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.

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 should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the Control Serum 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. Dilute each test serum 1/100 in Serum Diluent by adding 10µl of serum to 1000µl of Serum Diluent. DO NOT DILUTE THE CONTROLS.
5. Dispense 100µl of diluted sample or control serum into the appropriate wells. Gently shake for 5 seconds.
6. Cover the plate with the plate lid provided and place it on top of moist absorbent paper at 37ºC for 20 minutes.
7. 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.
8. 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. Wash the empty wells 3 times.
9. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. 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.
11. Dispense 100µl of Anti Human HRP IgG Conjugate into each well. Gently shake 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 37ºC for 20 minutes.
12. Wash plate as described above.
13. Dispense 100µl of stabilised Substrate Solution into each well. Gently shake for 5 seconds before replacing the lid and incubating in the dark at room temperature (20ºC to 30ºC) for 10 minutes.
14. 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. 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 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.

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 control serum and test serum, although not required, is recommended.

Control serum and test serum 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 for the Negative Control should be less than 0.2 the Low Positive Control should be between 0.3 and 0.7 and the High Positive Control should be greater than 0.8 for the assay results to be valid.
Cut off level for IgG = Average OD value of the duplicate low Positive Control.
For comparisons between assays antibody indexes (AI) must be calculated:

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

IgG
AI<1 is suggestive of no dengue infection. Re-testing is recommended after a few days.
AI>1 is suggestive of dengue infection.
AI $\geq 1 \leq 2$ is suggestive of primary infection.
AI>2 is suggestive of secondary infection where a large number of IgG antibodies can be detected after a few days from fever onset.
If levels of controls or users known samples do not give expected results, test results must be considered invalid.

PERFORMANCE DATA

In clinical studies, **PATHOZYME DENGUE G** shows excellent correlation with haemagglutination inhibition assay (HAI) which WHO recommends to identify Dengue fever. Full clinical data is available upon request.

EVALUATION DATA

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

	Pathozyme Dengue G		Totals
	Positive	Negative	
Positive	52	1	53
Negative	3	35	38
	55	36	91

Sensitivity 52/53 = 98.11%
Specificty 35/38 = 92.1%

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

REFERENCES

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QUICK REFERENCE TEST PROCEDURE

1. Dilute the test serum 1/100 in Serum Diluent by adding 10µl of serum to 1000µl of Serum Diluent.
2. Dispense 100µl of diluted sample or working strength control sera to each well. Gently shake for 5 seconds.
3. Incubate for 20 minutes at 37ºC.
4. Discard well contents and wash 3 times.
5. Dispense 100µl of Anti-human HRP IgG conjugate to each well. Gently shake for 5 seconds.
6. Incubate for 20 minutes at 37ºC.
7. Repeat step 4.
8. Dispense 100µl of substrate solution to each well. Gently shake for 5 seconds.
9. Incubate in the dark for 10 minutes at room temperature (20ºC to 30ºC).
10. Dispense 100µl of Stop Solution to each well.
11. Read the OD using an EIA reader with a 450nm filter.
12. Interpret results as described in the Interpretation of Results section

8069 ISSUE 4 Revised April 2006

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