

PATHOZYME[®] DENGUE M Capture Ref OD647

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 M CAPTURE kits is an enzyme-immunoassays (EIA) for the detection of 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 who visit these places. 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 M CAPTURE 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

IgM capture antibody is coated onto the surface of microtitration wells. Diluted test sera are then applied. All IgM antibodies in the sample are captured by the antibody in the wells. Unbound material is then washed away and a mixture of peroxidase conjugated Dengue antigens from all 4 serotypes is applied. If Dengue-specific IgM 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 IgM 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.

CONTENTS

Ref
OD647



| | |
|--|------------------|
| Microtitre Plate | 12 x 8 wells x 1 |
| Breakable wells coated with rabbit anti-human IgM antibodies 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 IgM antibodies to Dengue. Ready to use. (Blue) | |
| Control L | 2 ml |
| Low Positive Control. Clear solution, of human serum containing a low level of IgM antibodies to Dengue. Ready to use. (green) | |
| Washbuf 20X | 50 ml |
| Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless) | |
| DIL Conj | 13 ml |
| Conjugate Dilution Buffer. Phosphate based buffer containing stabilising proteins. Ready to use. (Purple) | |
| Conj 20 X | 0.65 ml |
| Dengue HRP Conjugate concentrate: Dengue antigens conjugated to Horseradish Peroxidase. (Colourless) | |
| 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 | 11 ml |
| 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: 20µl, 100µl, 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 M CAPTURE 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 disposal. Final disposal must be in accordance with local legislation.

PATHOZYME DENGUE M CAPTURE 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 CAPTURE 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 CAPTURE 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

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

SERUM DILUTION 1/100. This can be achieved by adding 10µl of serum to 1000µl of Serum Diluent.

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.

Conjugate:
For each strip being used, mix 1 ml Conjugate Diluent with 50 µl Dengue HRP Conjugate (20x). The diluted Conjugate is stable for 4 hours at room temperature (20°C to 25°C) or 24 hours at 4°C.

| No. of Strips (8 wells per strip) | Volume of Dilution Buffer | Volume of 20 X Concentrate Conjugate |
|-----------------------------------|---------------------------|--------------------------------------|
| 1 | 1 ml | 50 µl |
| 6 | 6 ml | 300 µl |
| 12 | 12 ml | 600 µl |

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. Seropidemiology 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

- Bring all the kit components and the test sera to room temperature (20° to 30°C) prior to the start of the assay.
- The kit Control Sera should be run with each batch of specimens to check the test performance and determine the cut off value. Kit controls should be run in duplicate. Select sufficient microwell strips for the number of samples to be tested including the Kit Control Sera. Locate the strips in the frame. Record the positions of the kit Control Sera and the test sera on the EIA Data Recording Sheet provided. Unused strips should be stored in the re-sealable foil bag, containing the desiccant, before being replaced at 2°C to 8°C for storage.
- Dilute each test sera 1/100 in Serum Diluent by adding 10µl of serum to 1000µl of Serum Diluent. DO NOT DILUTE THE CONTROLS.
- Dispense 100µl of diluted samples and control sera into the appropriate wells. The control sera 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 at 37°C for 60 minutes.
- 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.
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 or paper towel to remove all residual water droplets. Wash the empty wells 5 times.
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 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
- Dispense 100µl of working strength Conjugate into each well. Gently shake the plate for 5 seconds before replacing the lid onto the plate and returning to the 37°C incubator ensuring that the plate is positioned on top of the moist absorbent paper. Incubate at 37°C for 60 minutes.
- Wash plate as described above.
- Dispense 100µl of stabilised Substrate into each well. Gently shake for 5 seconds before replacing the lid onto the plate and placing in the 37°C incubator for 30 minutes.
- 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 Dengue IgM 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 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.

Cut off level = 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.

Assay Validation: The average OD's for the Negative Control should be less than 0.2 and the Low Positive Control should be greater than 0.20 for the assay results to be valid.

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 DENGUE M CAPTURE** is less than or equal to 10%.

| | Positive Result | Negative Result | Totals |
|---------------|-----------------|-----------------|------------|
| Positive sera | 116 | 1 | 117 |
| Negative sera | 2 | 63 | 65 |
| Total | 118 | 64 | 182 |

Total sensitivity 116/117 = 99.15%

Total specificity 63 /65 = 96.92%

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QUICK REFERENCE TEST PROCEDURE

1. Dilute the test sera 1/100 by adding 10µl of serum to 1000µl of Serum Diluent .
2. Dispense 100µl of negative control, sera and low positive control to a well. Gently shake for 5 seconds.
3. Incubate for 60 minutes at 37°C.
4. Discard well contents and wash five times.
5. Dispense 100µl of working strength conjugate to each well. Gently shake for 5 seconds.
6. Incubate 60 minutes at 37°C.
7. Repeat step 4.
8. Dispense 100µl of substrate to each well. Gently shake for 5 seconds.
9. Incubate in the dark for 30 minutes at 37°C.
10. Dispense 100µl of Stop Solution to each well.
11. Read the OD using an EIA reader with a 450nm filter.

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